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PATHOLOGY

A Periodical Devoted to General and Experimental Pathology

AMERICAN MEDICAL ASSOCIATION PUBLICATION

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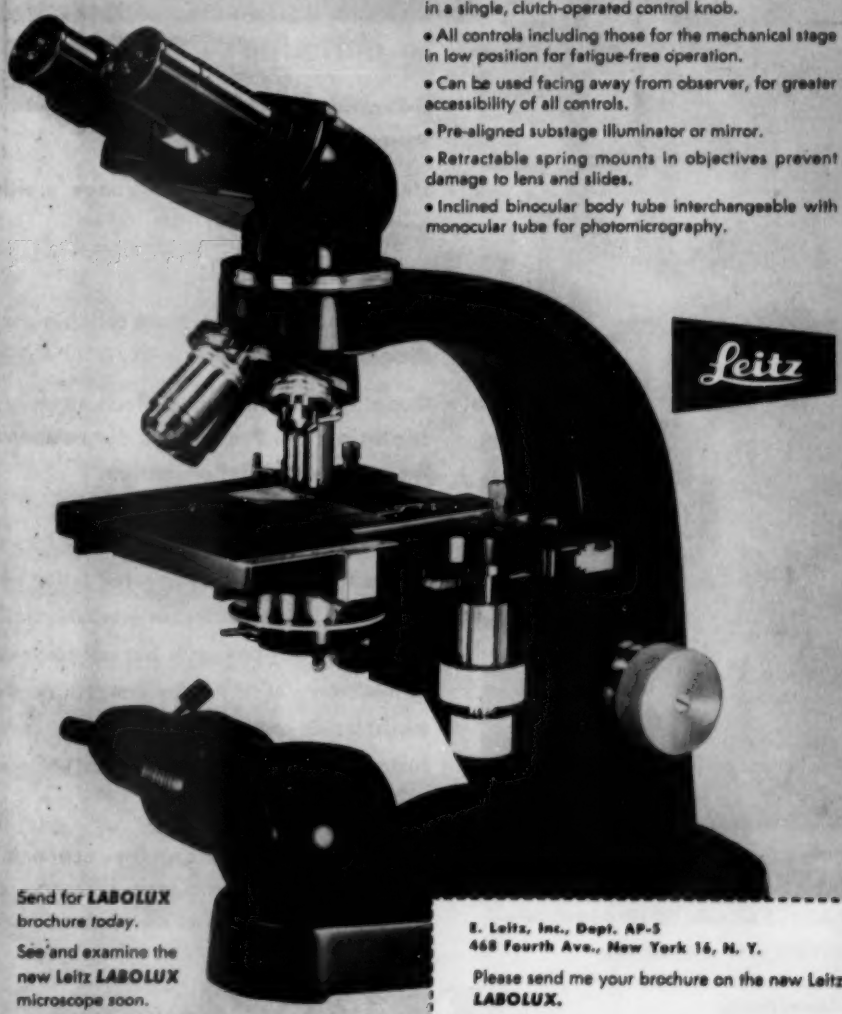

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A.M.A. ARCHIVES OF
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Role of Occupational and Environmental Air Pollutants in Production of Respiratory Cancers

W. C. HUEPER, M.D., Bethesda, Md.

1. The Problem and Its Scope

Environmental cancers, which, like infectious diseases, form a segment of the environmental disease panorama, are caused by specific chemical and physical agents, the majority of which have been introduced into the human environment during the last 100 years through the development of modern industry. The epidemiologic pattern associated with these health hazards, as well as some limited practical experiences obtained in the past with the containment of several occupational cancers, indicates that industrial and sanitary engineering methods can as effectively be applied toward the control of environmental cancers as they have been employed previously in the prevention of communicable diseases. Sanitary and industrial engineers have, for this reason, a special stake in the problem of environmental cancer hazards, which during the last decade have gained considerable stature as major causes of human cancers of various organs and tissues (Hueper).

A particular facet of this problem which for several reasons has attracted recently special attention is related to cancers of the respiratory organs, i. e., the lung, bronchi, trachea, larynx, nasal cavity, and paranasal sinuses. The majority of the occupational carcinogens discovered during the past 30 years produce cancers of the respiratory organs (Table 1). The remarkable and progressive rise in frequency of lung cancers, beginning about the turn of the century, has been accompanied by an increasing

Submitted for publication Dec. 14, 1956.

National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.

Presented before the Section on Engineering and Sanitation and the Conference of Municipal Public Health Engineers on Nov. 13, 1956, Atlantic City.

TABLE 1.—*Occupational Respiratory Carcinogens and Cancers Recorded During Past Seventy-Five Years; Their Causes, Sites, and Numbers*

Agent	Site of Cancer	Year Discovered	Number of Recorded Cases		
			United States	Other Countries	Total
Arsenic	Lung	1930	7	16	23
Asbestos	Lung	1934	22	74	96
Chromates	Lung	1932	75	65	140
Nickel	Lung	1932	0	84	84
	Nares and nasal sinus	----	0	51	51
Coal tar	Lung	1936	0	55	55
Petroleum oils	Lung and larynx	1936	7	33	40
Isopropyl oil	Lung	1946	1	0	1
	Larynx	----	4	0	4
	Nasal sinus	----	6	0	6
Radioactive chemicals	Lung	1879	0	025	025
	Nasal sinus	1931	3	0	3
Total			125	1,001	1,126

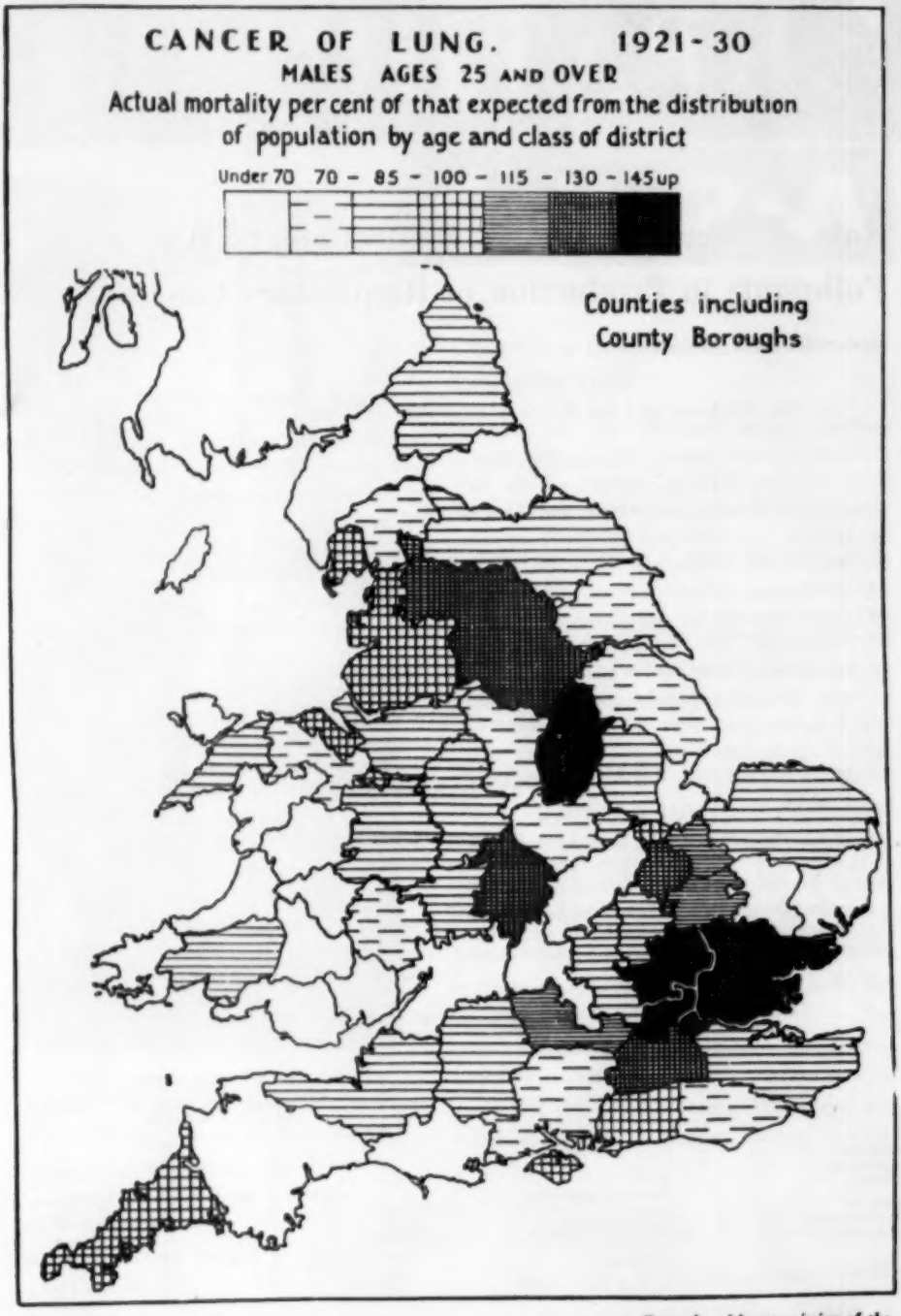


Figure 1

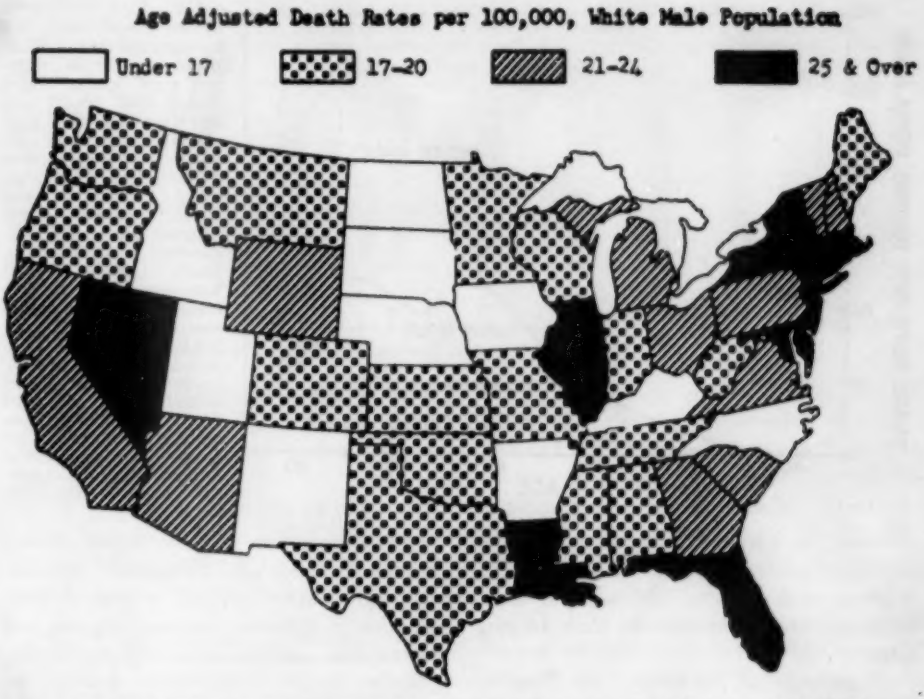


Fig. 2.—Respiratory system cancer, white males, United States, 1950. Age-adjusted death rates per 100,000 of the white male population. From Lew, E. A.: Cancer of the Respiratory Tract: Recent Trends in Mortality, *J. Internat. Coll. Surgeons* 24:12-27, 1955; published by the International College of Chicago, Chicago.

pollution of the atmosphere with industrial effluents and industry-related waste products, some of which possess established and strongly carcinogenic properties (Kotin et al.; Hueper). The relative direct or indirect causal role which occupational and general environmental air pollutants of industrial origin have assumed in the production of respiratory cancers and in their increased frequency has become, during recent years, a matter of debate, especially because of the various claims advanced concerning the predominating role of cigarette smoking in this respect (Doll; Clemmesen; Wynder and Graham; Hammond and Horn; Heady and Kennaway). The rather confused situation which has resulted from the various contradictory assertions in this matter is of immediate importance and calls for an early resolution because of the growing seriousness of the lung cancer problem and the urgency of developing a rational and balanced research and control program based on the entire factual evidence obtained from all pertinent sources and on a global scale, and competently interpreted. Without such clarification neither can the scope and character of experimental research to be devoted to the various etiologic aspects of lung cancers be determined rationally nor can intelligent decisions be arrived at concerning the extent and nature of prophylactic and preventive technologic and sanitary measures indicated, the sociologic adjustments to be made, the economic implications to be considered, and the legal and medicolegal consequences to be faced.

2. Epidemiologic Pattern

A critical analysis of the peculiarities of the epidemiologic patterns of the cancers

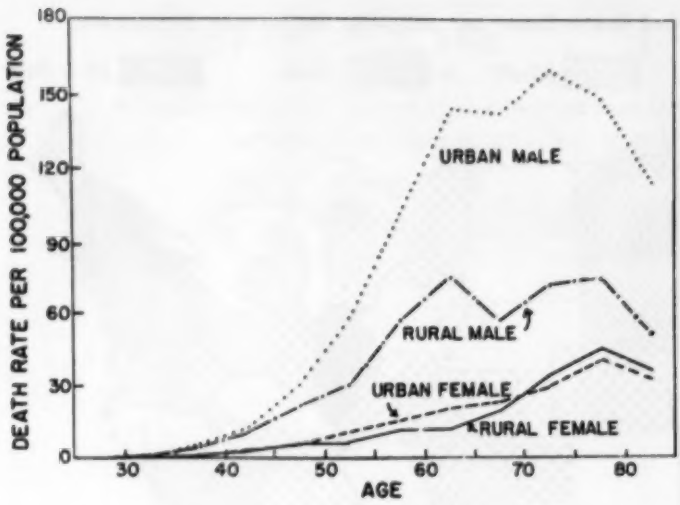


Fig. 3. — Respiratory system cancer death rate by age and sex. White population, urban states and rural states, United States, 1949. States with less than 40% urban population called rural, and states with more than 80% urban population called urban. Rural states: Vermont, North Dakota, Mississippi, Arkansas, North Carolina, South Carolina, West Virginia, and Kentucky; urban states: Rhode Island, Massachusetts, New York, New Jersey, California, District of Columbia. Source: Vital Statistics of the United States, 1949, 1950; Population Census of the United States; Statistical Research Section, American Cancer Society, 1949.

affecting the different parts of the respiratory tract provides some circumstantial evidence as to the general nature of the cause or causes which may underlie their varying behavior.

A. Increase of Frequency; Its Time of Onset; Its Regional Distribution.—Deaths from cancer of the lung have increased progressively during the last five decades in practically all countries of the Western World, where well-developed medical care and records are available (Dunn; Dorn; Dorn and Cutler; Hueper; Kotin; Lickint; Kahlau; Weller; Doll; Pascua).

The epidemiologically and, therefore, also etiologically significant and outstanding feature of this development is its marked irregularity as to time of onset, degree of rise, progression rate, sex distribution, and annual and relative increase in tobacco consumption in different countries, regions, and communities, and its consistent association with the degree of population density, industrial activities, urban and rural areas and population groups, and the industrial and industry-related pollution of the atmospheric air with known and highly potent respiratory carcinogens.

First discovered in several large industrial cities of Saxony around the turn of the century, the increase in lung cancer frequency was subsequently demonstrated in almost all countries of Europe and North America (Ringertz). However, this devel-

TABLE 2.—*Increase in Lung Cancer Deaths in Different Countries: Rates per 100,000 Deaths in Males* *

Country	Year and Crude Lung Cancer Death Rate					
	Year	Rate	Year	Rate	Year	Rate
England and Wales	1930–1932	12.9	1949	49.5	1952	61.4
Scotland	1930–1932	10.6	1949	41.4	1952	56.3
Finland	1936–1938	13.6	1949	29.8	1952	38.0
Switzerland	1929–1931	12.0	1949	26.1	1952	33.5
New Zealand	1930–1932	7.1	1949	21.6	1952	31.5
Netherlands	1929–1931	7.2	1949	24.5	1952	30.5
France			1949	21.7	1952	28.2
United States	1929–1931	4.3	1949	21.5	1952	26.1
Denmark	1934–1936	4.5	1949	16.7	1952	24.8
Ireland	1935–1937	3.4	1949	15.1	1952	22.2
Australia	1933–1934	6.3	1949	16.7	1952	20.8
Canada	1930–1932	4.2	1949	16.4	1952	19.0
Italy	1931	3.0	1949	11.3	1952	16.4
Norway	1929–1931	1.8	1949	9.0	1952	11.5
Japan	----	----	1949	3.3	1952	4.9

* Compiled from data published by P. Kotin (*Cancer Res.* 16: 375-393, 1956).

TABLE 3.—*Increase in Lung Cancer Deaths in Different Countries: Rates per 100,000 Deaths in Females* *

Country	Year and Crude Lung Cancer Death Rate						1952 Position	1930 Position
	Year	Rate	Year	Rate	Year	Rate		
England and Wales	1930–1932	4.3	1949	9.8	1952	11.3	1	2
Scotland	1930–1932	5.6	1949	10.8	1952	10.9	2	1
Finland	1936–1938	2.3	1949	5.4	1952	6.5	4	6
Switzerland	1929–1931	2.0	1949	4.7	1952	4.7	10	9
New Zealand	1930–1932	2.5	1949	3.9	1952	5.1	9	4
Netherlands	1929–1931	2.2	1949	4.4	1952	4.0	13	8
France			1949	5.6	1952	6.1	6	...
United States	1929–1931	1.9	1949	4.9	1952	5.4	7	11
Denmark	1934–1936	2.5	1949	4.3	1952	6.2	5	5
Ireland	1935–1937	3.2	1949	4.5	1952	7.3	3	3
Australia	1932–1934	2.3	1949	3.6	1952	4.3	12	7
Canada	1930–1932	2.0	1949	4.6	1952	4.0	14	10
Italy	1931	1.4	1949	3.4	1952	4.5	11	12
Norway	1929–1931	1.2	1949	3.6	1952	5.4	8	13
Japan			1949	1.2	1952	2.2	15	...

* Compiled from data published by P. Kotin (*Cancer Res.* 16: 375-393, 1956).

opment varied distinctly in its time of onset, its progression rates, its male-female sex ratio, and its degree in different countries and regions (Hueper; Kotin) (Tables 2 and 3). For instance, a rise in lung cancer deaths became first noticeable in 1930 for Danish males, but was restricted at that time to males residing in Copenhagen. It was not until 10 years later that the same phenomenon was observed for males living in rural areas and small towns of Denmark (Clemmesen and Nielsen).

TABLE 4.—*Lung Cancer Death Rates in Metropolitan, Urban, and Rural Counties of Ohio* *

Metropolitan counties (8)	122.9
Urban counties (7)	81.8
Rural counties (73)	68.6
The standard mortality ratio is $\frac{\text{Observed deaths}}{\text{Expected deaths}} \times 100$	

* Compiled from data by Mancus et al. (*Am. J. Pub. Health* 45:58-70, 1955). The type of county is defined, according to degree of urbanization, as follows: metropolitan county, containing cities with 1950 populations of 100,000 or more (91% urban); urban, containing cities with 1950 populations of 50,000-1,000,000 (66.2% urban); rural, containing communities with 1950 populations below 50,000 (41.4% urban).

Similarly irregular, if not erratic, patterns of lung cancer death rates for both sexes were reported for subdivisions (states, provinces, metropolitan areas, and sections of metropolitan areas) of several countries (Figs. 3, 4, 5, 6, and 7; Tables 4, 5, and 6) (Mancuso, Macfarlane, and Porterfield; Herbich and Neubold; Stocks; Griswold; Lew; Hoffman and Gilliam; Patno; Gilliam; Haenszel, Marcus, and Zimmerer; Cruickshank; Wilder).

TABLE 5.—*Distribution, Expressed in Per Cents, Areas of Connecticut 1947-1950* *

Area Sex	Heavy Industry	Textile-Paper		Agriculture Control
		Urban	Rural	
Males	50.6	51.5	41.2	34.3
Females	9.8	8.6	11.4	6.8

* Compiled from data published by C. S. Wilder (*Connecticut Health Bull.* 70:1-8, 1956). See also Figure 5.

Area 1 of Pittsburgh (Fig. 7), which showed excessive prevalence rates for cancers of the respiratory organs and of the skin for white men, consists of the highly polluted "downtown" and "hill" sections with residents from the bottom of the socio-economic scale (Patno). The respiratory and cutaneous cancer rates in this district were practically twice those for men living elsewhere in the city, and the probability that the difference was due entirely to sample variations was less than 0.001. It is significant that many occupational agents (coal tar, petroleum oils, arsenicals, radioactive substances) causing cancers of the skin elicit, when inhaled, cancers of the lung.

TABLE 6.—*Lung Cancer Mortality Rates, per 1000 Deaths by Sex, in Austria, 1954* *

Community	Total	Males	Females
Vienna	32.7	59.0	7.8
Cities 60,000-1,000,000	18.2	31.6	5.0
Cities 20,000-60,000	18.4	32.3	3.9
Remainder of Austria	10.3	17.3	3.7

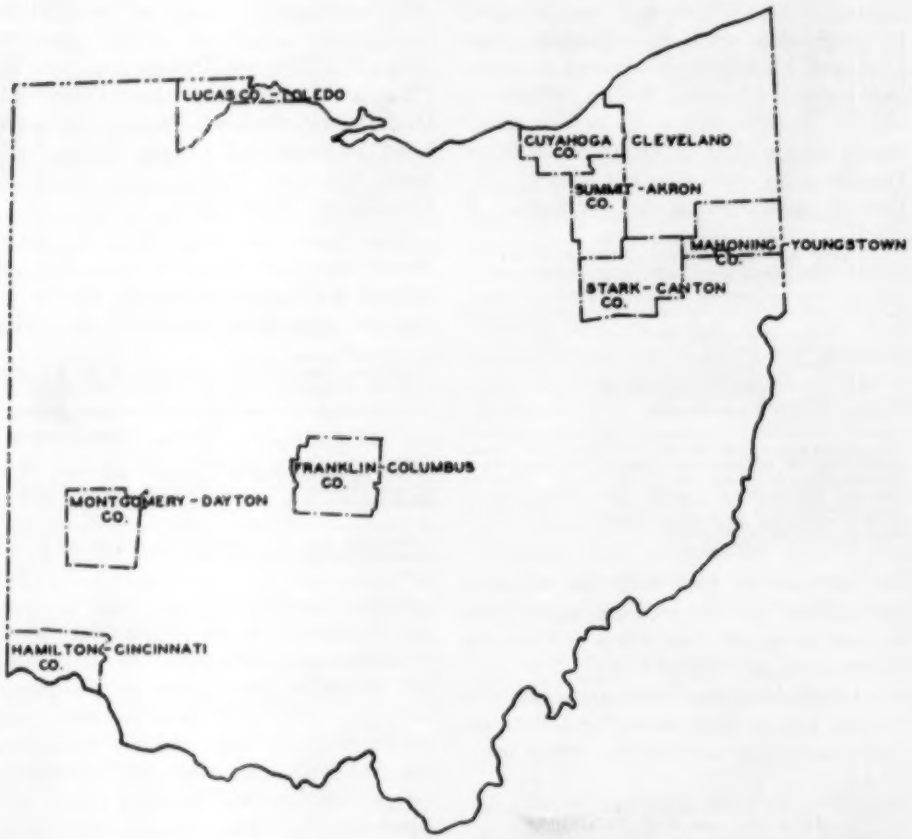
* Compiled from data published by Herlich and Neubold (*Ztschr. Krebsforsch.* 60:139-160, 1954).

While, as a rule, urban and industrialized and densely populated regions have decided-

ly higher lung cancer rates than rural areas (Curwen, Kennaway, and Kennaway; Doll; Stocks; Clemmesen, Nielsen, and Jensen; Gilliam; Dorn), there occur not only marked fluctuations in this respect among communities of similar size and type (Table 8), but also exceptions to the rule. The lung cancer rates of several metropolitan areas in Ohio, for instance, were not excessive. Kreyberg also recently stated that in Norway some cities with industries and industrial centers did not show higher figures than do smaller cities with "pure" air.

The epidemiologic data recorded indicate that whatever factors were introduced into the human environment some 75 years ago, and caused a rise in lung cancer frequency during the last 50 years, did not become active in all countries, regions, and communities at the same time and to the same degree, although male inhabitants of urban areas were most strongly affected by them in most instances.

B. Environmental Causal Agents.—An examination of the human environment for factors which fulfilled the above-listed epi-



	OBSERVED	EXPECTED	POPULATION
8 METROPOLITAN COUNTIES	1550	1261.1	9 208 500
BALANCE OF OHIO	728	1016.9	7 424 300

Fig. 4.—Observed and expected lung cancer deaths in urban and rural Ohio 1947-1951. From data in Mancuso et al. *Am. J. Pub. Health* 45:58-70, 1950; published by the American Public Health Association, New York.

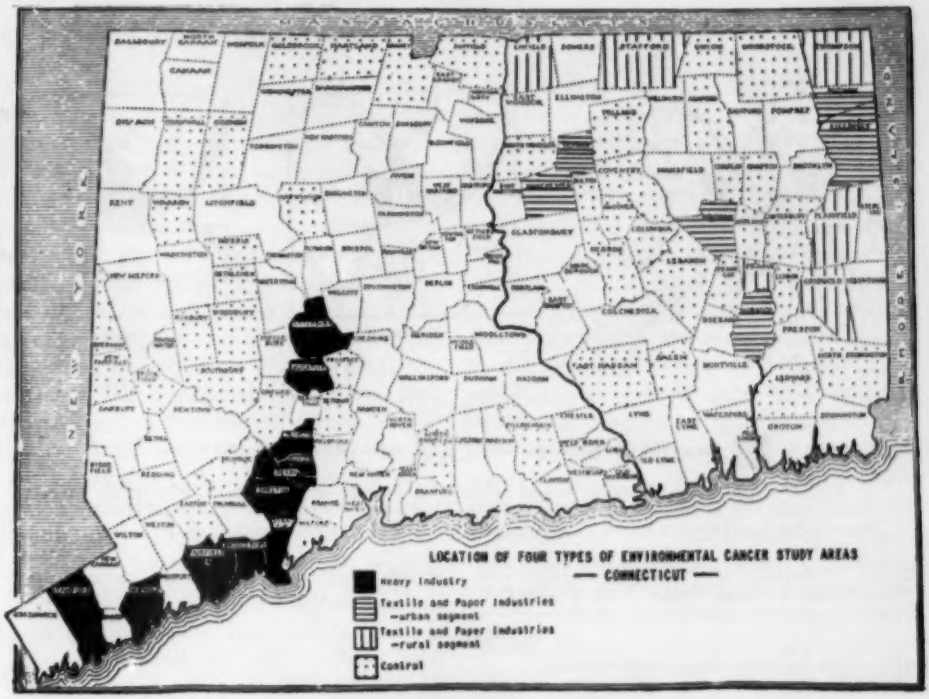


Figure 5

From Wilder, C. S.: *Connecticut Health Bull.* 70:1-8, 1956; published by the Connecticut State Board of Health, Hartford.

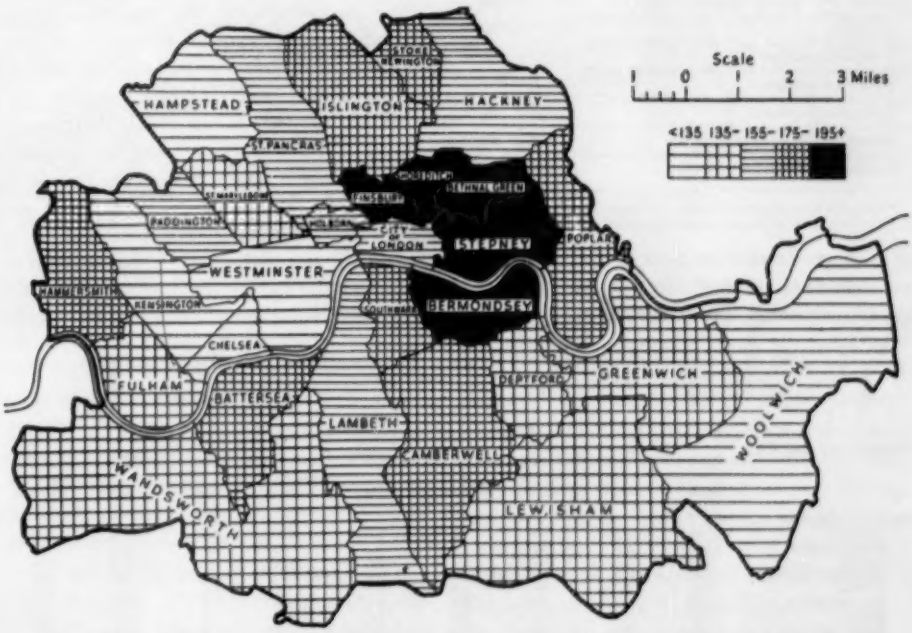


Figure 6.—Cancer of lung, bronchus, and pleura. 1946-1949 deaths of males per 100 of male population (calculated by applying rates for England and Wales to populations at ages 0-, 35-, 45-, 55-, 65-, 75+) in the City of London and metropolitan boroughs. From Stocks, P.: *Brit. J. Cancer* 6:99-111, 1952; published by the British Empire Cancer Campaign.

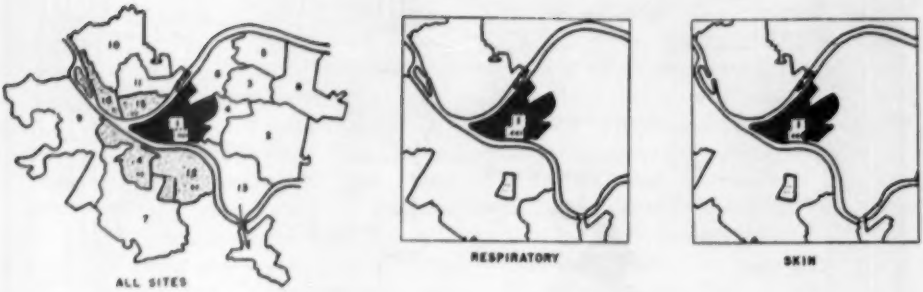


Fig. 7.—Prevalence of cancer among white men, by site, in 16 areas of Pittsburgh, 1947. From Patno, M. E.: *Pub. Health Rep.* 69:705-715, 1954, p. 709; published by the U. S. Public Health Service, Department of Health, Education, and Welfare.

TABLE 7.—*Relation of Density of Population in Conurbations to Lung Cancer Rates 1946-1949* *

Groups of adjacent towns with over 200,000 occupied dwellings	Mortality Rate†
London, East Ham, West Ham, Croydon	156
Birmingham, Smethwick, Walsall, West Bromwich	134
Manchester, Salford, Stockport	159
Liverpool, Bootle, Birkenhead, Wallasey	162
Leeds, Bradford, Halifax	132
Sheffield, with 124,000 occupied dwellings	135
Newcastle and Gateshead, with 87,000 occupied dwellings	114
Aggregate of 6 towns, each with 50,000 to 85,000 occupied dwellings	113
Aggregate of 3 towns, each with 40,000 to 50,000 occupied dwellings	107
Aggregate of 12 towns, each with 30,000 to 40,000 occupied dwellings	104
Aggregate of 13 towns, each with 20,000 to 30,000 occupied dwellings	100
Aggregate of 29 towns, each with less than 20,000 occupied dwellings	80

* Adapted from P. Stocks (*Brit. J. Cancer* 6:99-111, 1952).
† Per cent ratio of registered to calculated deaths.

demiologic conditions, and at the same time possessed established or possible carcinogenic properties, brought two main groups of substances under suspicion, namely, occupational and industry-related air pollutants of various types and constituents of cigarette smoke. The absolute and relative importance of these two groups of environmental agents in the causation and increase in frequency of lung cancer has become the subject of widely varying appraisals.

The supporters of the cigarette theory claimed that the various statistical correlations obtained between the relative liability to lung cancer and cigarette smoking also extended to the urban-rural distribution pattern of these tumors, since urban populations indulged more heavily in this habit than rural ones (Doll; Hammond and Horn; Doll; Kreyberg; Haenszel and Shimkin). Doll asserted, in fact, that this factor accounted for the entire difference between urban and rural lung cancer rates. Apart

TABLE 8.—*Incidence of Respiratory Cancer; Morbidity Rates per 100,000 Population for Nine Metropolitan Centers by Sex, 1937 and 1947*

Primary Site and City	Morbidity Rates								
	Males			Females			Total		
	1937	1947	Per Cent Increase	1937	1947	Per Cent Increase	1937	1947	Per Cent Increase
Bronchus and lung									
Atlanta	5.0	13.4	168	1.0	5.0	400	2.9	8.9	207
New Orleans	13.1	39.1	198	2.8	4.2	50	7.6	20.8	174
Dallas	5.9	29.0	392	0.5	6.4	1180	3.1	17.2	455
Birmingham, Ala.	4.5	18.9	320	2.1	3.9	86	3.3	11.0	233
Denver	9.1	21.9	141	4.2	8.1	93	6.6	14.8	124
San Francisco	15.6	34.3	120	3.9	8.1	108	9.8	20.5	112
Chicago	13.3	29.5	122	4.3	7.0	63	8.8	18.0	105
Pittsburgh	9.7	26.1	169	4.9	5.5	12	7.3	15.6	114
Detroit	12.6	32.0	154	2.3	5.7	148	7.6	19.0	150

from the fact that some of the data used in these calculations are of dubious scientific value, the markedly irregular epidemiologic pattern of lung cancer for various regions and population groups associated with exposures to industry-related air pollutants of widely different types and degree practically excludes the possibility that one single factor is involved in being responsible for the wide variations in incidence rates. Lung cancer is definitely not a disease entity, like smallpox, but is a group of diseases with different and distinct causal factors. Statistical-epidemiological considerations applicable to smallpox therefore have no place in the interpretation of statistical data concerning lung cancer, and cannot justly be advanced in support of the allegation that some 80% to 95% of all lung cancers in men are due to cigarette smoking. Experienced biostatisticians recently have called attention to the common statistical fallacy of ascribing cause-and-effect relationship to an association by rationalization (Arkin; Dorn; Berkson; Macdonald). The possibility of misinterpretations of statistical data obtained from a single factor study of a disease having recognized multiple causal factors of wide distribution, marked carcinogenic potency, and relations as to time of introduction and increase of exposure similar to those of the specific factor analyzed appears to be sufficiently great in this particular instance for cautioning against sweeping conclusions. It would be well to remember in this connection that some statisticians who now advocate strongly the validity of the cigarette theory up to a few years ago were equally confident on the basis of their statistical data that severe trauma is one of the factors in the etiology of breast cancer (Lane-Claypon; Lombard), although the concept of an acute traumatic genesis of cancer has belonged for some time to those concepts of carcinogenesis having no credit among competent oncologists (Hueper). Perhaps Stocks and Campbell had such facts in mind when they proposed recently a much more conservative estimate regarding the role of cigarette smoking in the production of lung cancer among the inhabitants of North Wales and the Liverpool region, since they attributed only about 50% of the cases observed in the Liverpool area to cigarette smoking, while three-fourths of the remaining cases were thought to be caused by an "urban" factor, presumably industrial air pollutants. However, even this estimate is heavily biased by the arbitrary assumption that the benzpyrene content present allegedly in cigarette smoke was about 12 times as effective in eliciting lung cancers as benzpyrene demonstrated in atmospheric air. Only when such a "corrective" coefficient is applied was it possible to obtain proportional correlations between the total exposure to benzpyrene from both cigarette smoking and air pollutants and the relative incidence rates of lung cancer found in the industrialized metropolitan Liverpool area, an intermediary urban-rural region, and the rural area of North Wales. As one of the surprising by-products of this weighted calculation, it appears that the great majority of lung cancers occurring among the rural population group are attributable to cigarette smoking, although farmers also have contact with arsenical insecticides and sheep-dip, soot from domestic coal furnaces, and the exhaust of gasoline and Diesel engines used for cultivating purposes.

Yielding to a tendency of generalization and oversimplification in the extension of observations made on a restricted or selected scale, proponents of the cigarette theory do not take proper cognizance of the facts that the rise in lung cancer rates started before cigarette smoking assumed large proportions, and that no consistent relation exists between the consumption of tobacco and cigarettes in different countries and during various periods and their respective lung cancer rates (Daff, Doll, and Kennaway; Doll; Herbich and Neubold; Denk; Moore; Rigdon and Kirchoff; Llombart). Although the male lung cancer death rate for England and Wales is twice that of the United States,

TABLE 9.—*Observed and Expected Mortality* from Cancer of Lung According to Place of Birth, New Zealand or the United Kingdom†*

Place of Birth	Observed Deaths	Expected Deaths	Age at Entry to United Kingdom			
			Under 30		30 and over	
			Observed Deaths	Expected Deaths	Observed Deaths	Expected Deaths
New Zealand	632	721.8	.....	.....	.....	.....
United Kingdom	369	279.9	201	229.2	168	139.8

* The significance of the difference between observed and expected deaths in the two countries is $p<0.001$.
† Adapted from D. F. Eastcott (*Lancet* 1:37-39, 1956). Eastcott suggested that the higher incidence of lung cancer among British immigrants might be due to an exposure to industrial air pollutants sustained in Great Britain previous to their entrance into New Zealand.

the per capita consumption of cigarettes in the United States is 30% higher than in England and Wales. Gsell did not find any significant differences in the smoking habits between urban and rural physicians in Switzerland, showing thereby that, for at least this instance, the allegedly existing urban-rural ratio of cigarette smoking does not apply.

Recent studies of Eastcott on the relation of cigarette consumption and lung cancer liability also show that this claimed association is not demonstrable for the inhabitants of New Zealand. Eastcott found that settlers from Great Britain are more liable to lung cancer than persons born in New Zealand, who smoke just as much (Table 9).

A similar connotation must be attached to the results of recent studies of Cohart concerning the socioeconomic distribution of lung cancer in New Haven, Conn. The incidence of lung cancer was found to be about 40% greater among the poor than among the other socioeconomic classes. Unless it is assumed, according to Cohart, that cigarette smoking is inversely related to socioeconomic status, an assumption that probably cannot be supported in fact, then it is reasonable to conclude that important environmental factors other than cigarette smoking exist that contribute to the causation of lung cancer. Again it is significant concerning the irregularity of the epidemiologic lung cancer pattern that socioeconomic conditions do not seem to exert any demonstrable influence upon lung cancer rates of various classes, indicating that the mortality gradient observed between the social classes in England may be due to environmental differences which have no parallel in the United States (Hewitt and Brooksbank).

The obvious importance of local environmental carcinogens of probably industrial nature other than tobacco smoke for the production of lung cancer and for the determination of incidence rates which is evident from observations made in Connecticut (Table 5) and Pittsburgh (Fig. 7), receives support through the observations made in Montana by Lull and Wallach, who noted excessive lung cancer death rates in counties with special industrial operations (Table 10).

The total qualitative and quantitative epidemiologic evidence on the regional distribu-

TABLE 10.—*Lung Cancer Mortality in Several Montana Counties, 1947-1948**

County and Total Population 1950	Major Industry	No. Lung Cancers		Total	Total Cancer Deaths	Per Cent Lung Cancer		Annual Lung Cancer Death Rate/100,000	
		Male	Female			Male	Female	Male†	Female
Deer Lodge, 13,627	Copper smelting‡	21	0	21	98	30.8	0.0	145.7	0.0
Silver Bow, 53,207	Copper mining‡	27	2	29	259	22.6	1.5	48.6	3.9
Cascade, 41,499	Copper mining, smelting‡	20	5	25	299	12.7	3.5	46.3	12.3
Gallatin, 18,269	Agriculture	1	0	1	81	3.0	.0	5.2	.0

* Lull and Wallach: Personal communication of unpublished data.
† The estimated crude death rate for lung cancer among white males in the entire United States in 1947 was 10.9 per 100,000 population.
‡ The workers employed in copper ore mining and smelting inhale dust and fumes of arsenic contained in the ore and released as a by-product and waste product during the smelting process.

TABLE 11.—*Sex Distribution of Lung Cancer in the United States and Selected Foreign Countries, 1850-1953*

Country	Year	Author	Male-Female Ratio
United States	1953	Dorn	5:1
	1951	Moore	6.6:1
	1947	Humphreys	7:1
	1950	Beeler et al.	11.5:1
	1946	Lindskog	4.5:1
	1951	Carlisle et al.	20:1
	1941	Halpert	14:1
	1951	McBurney et al.	20:1
	1949	O'Keefe	20:1
	1941	Farberow and Baalow	13.5:1
	1935	Neely	1:1
Mexicans *	1953	Steiner	1:0.7
Norway	1953	Kreyberg	4:1
	1925	Kreyberg	1:1
Sweden	1947	Henschen	2:1
Denmark	1931	Clemmesen	5:4
	1945	Clemmesen	3:1
Austria	1953	Denk	15:1
Germany	1953	Grosse	6.6:1
	1850-1899	Grosse	1.8:1
	1900-1919	Grosse	3.1:1
	1920-1929	Grosse	3.8:1
	1930-1939	Grosse	3.8:1
	1940-1949	Grosse	5.4:1
France	1952	Lemoine	10:1
Canada	1948	Gagnon	8:1
Argentina	1947	Santas	50:1
England	1949	Mason	10:1
	1949	Fulton	7.3:1

* Mexicans living in Los Angeles.

tion of lung cancers supports the view that environmental factors other than cigarette smoke most likely account for the causation of these tumors and their recent increase in frequency.

C. Differences and Variations in Male-Female Sex Ratio.—Great importance has been attached by supporters of the cigarette theory to the fact that the increase in lung cancer has been much more marked among male than among female patients, that this development paralleled the increasing greater use of cigarettes by males, and that for this reason the two phenomena were causally interrelated. A critical analysis of the actual evidence reveals that such a conclusion can only be reached if the considerable fluctuations in the sex ratio found in different countries, among different population groups, and at different periods, are totally disregarded (Tables 11 and 12), and when no account is taken of the considerable amount of direct and circumstantial evidence pointing to a significant role of various chemical environmental air pollutants and differences in the degree of occupational and environmental exposure of members of the two sexes to these factors (Hueper; Kotin). Men work much more frequently and longer than women in occupations with known or suspected respiratory cancer hazards. They spend longer periods in urban environments, while women often spend most of their time in the cleaner suburbs,

TABLE 12.—*Male-Female Sex Ratio of Lung Cancers in Germany, 1886-1927 and 1940-1950*

1886-1927			1940-1950		
City	Author	Ratio	City	Author	Ratio
Dresden	Wolf	6.7:1	Dresden	Lickint	15:1
Leipzig	Seyfarth	5.3:1	Leipzig	Knorr	11:1
Leipzig	Sehrt	2.5:1	Leipzig	Merkel	18:1
Chemnitz	Briese	2.9:1	Zwickau	Gerbe	7:1
Berlin	Wahl	3.7:1	Berlin	Berg	9:1
Berlin	Bejach	2.3:1	Potsdam	Hollmann	19:1
Berlin	Hanf	3.6:1	Koeln	Breyer	21:1
Berlin	Redlich	5.2:1	München	Anacker	7:1
Koehn	Eichengruen and Essen	4.7:1	München	Kautzsch	18:1
München	Fuchs	1.5:1	München	Frey	24:1
Jena	Bilz	8.0:1	Jena	Kuntzen	49:0
Hamburg	Kikuth	1.8:1	Hamburg	Letzius	12:1

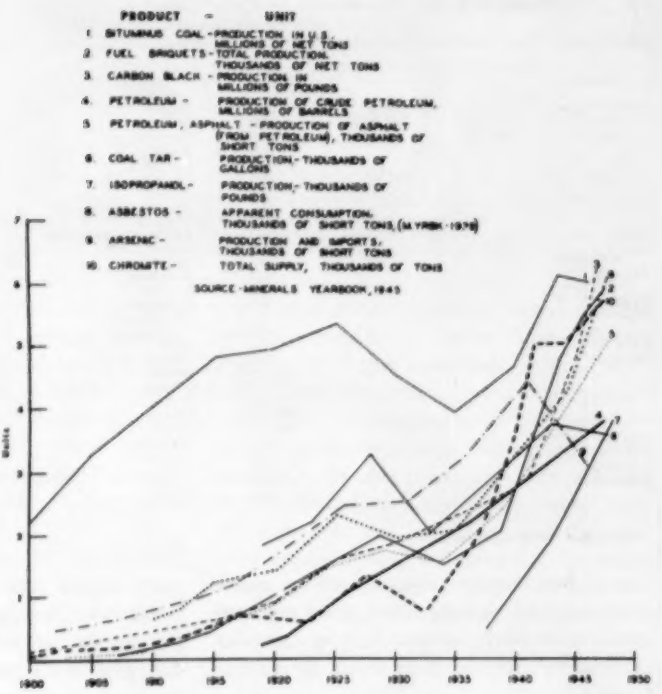


Fig. 8.—Rise of annual production or consumption of cancer-related industrial chemicals between 1900 and 1948.

and indoors, where they do not become exposed to the same degree as men to the general environmental air pollutants related to industrial activities and motor traffic. Whenever the type and degree of contact with respiratory carcinogens for occupational or environmental reasons tended to become equalized, the sex ratio revealed a similar tendency (asbestosis cancer of the lung [Hueper; Merewether]; domestic soot cancer of the lung in Mexican women [Steiner, Butt, and Edmondson]). These observations supply sound and serious reasons in support of the view that specific and general air pollution factors evidently are of considerable significance in determining sex ratios of lung cancer in different regions and populations, especially since some evidence exists indicating that the male-female sex ratio may be high (16:2), although women are heavy smokers (Correa).

TABLE 13.—*3,4-Benzpyrene Content of the Air of Eight English Cities* *

City	Mean Annual Concentration γ of 3,4-Benzpyrene in 100 Cubic Meter of Air
London (County Hall)	4.6*
Sheffield	4.2
Leicester	2.9
Burnley	2.7
Bilston	2.7
Cannock	1.9
Hull	1.8
Bristol	1.3

* The air was collected 75 ft. above ground level, and the results, therefore, are not directly comparable with the others, in which the air filtrate was obtained at lower levels or at ground levels.

† Adapted from Waller, R. E.: *Brit J. Cancer* 6:8-21, 1952.

3. Type, Quantity, and Quality of Air Pollutants.—During the last decades increasing amounts of industrial effluents from industries handling or producing cancer-related chemicals and of exhausts of motor vehicles have been released into the atmosphere (Fig. 8).

Data available from chemical analysis of air pollutants of metropolitan and industrialized regions provide additional evidence in this direction. Waller, determining the 3,4-benzpyrene content of air filtrates of

eight English cities, noted the mean annual concentrations of benzpyrene in the air shown in Table 13.

In commenting on the possible significance of these figures in terms of human exposure, Waller pointed out that they reflect only conditions close to each collecting station, and not necessarily those in the town as a whole. Marked seasonal variations in the benzpyrene content were observed, the highest values being found during the winter months, up to 15γ to 30γ per 100 cubic meters of air in Salford (Cooper), particularly during periods of smog, when the benzpyrene values rose fourfold above the usual level (32.8γ, as against 6.4γ to 9.8γ [Waller]). Similar local variations in the benzpyrene content of the air were reported from the Liverpool region by Stocks and Campbell, who recorded 0.7γ in 100 cubic meters of air for the rural region, 7.7γ in Liverpool, and 8.5γ in Bootle, giving a ratio of rural to urban atmospheric benzpyrene of 1:10.

In any attempt to apply these benzpyrene values to the human cancer problem as an index of the degree of exposure to carcinogenic air pollutants, proper consideration must be given to the fact that 3,4-benxpyrene is merely one of several carcinogenic hydrocarbons formed during the incomplete combustion of carbonaceous fuel (coal, oil, gasoline) (Shubik; Kotin), and therefore provides mainly an indicator for the presence of carcinogenic hydrocarbons in the air,

TABLE 14.—*Estimated Amount* of Aromatic Hydrocarbons in One-Minute Samples of Gasoline Exhaust with Varying Engine Revolution Speeds†*

Revolutions per Minute	Pyrene	Compound X	Benzpyrene	Benzperylene	Anthanthrene
500	225	289	120	235	153
1,000	430	325	61	177	102
1,500	507	266	33	60	36
2,000	374	142	40	73	27
2,500	346	127	25	70	31
3,000	121	25	13	85	14
3,500	48	5	10	39	15

* Quantities are expressed in micrograms at 0 load.
† Adapted from Kotin, Falk, and Thomas (*A. M. A. Arch Indust. Hyg.* 9:164-177, 1954).

but not an accurate or approximate measure of their amounts and relative carcinogenic potency. The observations of Kotin, Falk, Madar, and Thomas, and of Falk and Kotin, moreover, have demonstrated that the chemical nature of atmospheric carcinogenic pollutants depends in part upon the fuel burned. In an atmosphere polluted mainly by combustion products of coal, the air contains, in addition to various carcinogenic polycyclic aromatic hydrocarbons, small amounts of arsenic and radioactive matter originally present in the coal (Meetham; Goulden, Kennaway, and Urquhart; Anderson, Mayneord, and Turner) (6γ of As_2O_3 per 100 cubic meters).

Determinations made on the benzpyrene content of the particulate phase of gasoline and Diesel engine exhausts have shown that gasoline motors under traffic conditions prevailing in congested cities, and that Diesel engines when placed under strain or in

TABLE 15.—*Estimated Amount* of Aromatic Hydrocarbon in One-Minute Samples of Diesel Exhaust with Varying Load and Engine Revolution Speed and with Fuel Injection Inefficiency†*

Revolutions per Minute	Load	Condition	Pyrene	Compound X	Benzpyrene	Benzperylene	Anthanthrene
1000	0	Compression release	137	22	146	22	0
	1/4	Compression release	297	76	465	42	43
	1/2	Compression release	536	175	772	124	223
	3/4	Compression release	1800	640	1320	610	472
	4/4	Compression release	2500	639	876	1205	460
1200	0	Compression release	208	0	9	79	4.3
	1/4	Compression release	257	0	47	40	24
	1/2	Compression release	448	278	437	171	197
	3/4	Compression release	888	488	432	930	320
	4/4	Compression release	1912	614	1706	976	944
1400	0	Compression release	188	0	80	0	20
	1/4	Compression release	177	56	78	0	16
	1/2	Compression release	220	76	1372	368	69
	3/4	Compression release	734	337	982	1071	577
	4/4	Compression release	822	346	1687	944	666

* Quantities are expressed in micrograms per minute.
† Adapted from Kotin, Falk, and Thomas (*A. M. A. Arch. Indust. Hyg.* 9: 164-177, 1954).

improper working condition, generate appreciable amounts of 3,4-benzpyrene (Tables 14 and 15) (Kotin and associates; Lyons; Commins, Waller, and Lawther; Fitton; Mittler; Clemo et al.; Tebbens, Thomas, and Mukai).

Exhausts of gasoline motors, as well as volatilized gasoline from tanks, discharge uncombusted aliphatic hydrocarbons into the air, which are converted through the action of ozonoids into unsaturated and polymerized aliphatic compounds possessing carcinogenic properties to mice (Kotin; Kotin, Falk, and Thomas).

It is of special importance in relation to the human lung cancer problem that both aromatic and aliphatic carcinogenic hydrocarbons from motor vehicles are released into the air at ground level, where they can act upon pedestrians and drivers before they become highly diluted. Observations made on the carbon monoxide content of the air originating from motor cars at various heights above street level indicate that the concentration of the exhaust decreases rapidly with increasing height, being about one-third of the ground-level rate at 100 ft. (Fitton). The degree of exposure of urban residents and workers to carcinogenic components of motor exhaust, therefore, must vary considerably in different localities, at different heights above street level, and at different times, depending on the density of motor traffic, its speed, and its continuity, as well as on the dispersal rate of the exhaust, as determined by the height of the buildings and prevailing climatic conditions (sunshine, wind, rain, fog, temperature inversion).

In an appraisal of the relative and potential importance of these atmospheric pollutants for the human lung cancer problem, some guidance is provided by the administration of the various organic fractions obtained from filter residues of air to experimental animals. Cancers were produced consistently and in an appreciable number of mice painted and/or subcutaneously injected with ether extracts of household chimney soot by Passey (1922) and by Campbell (1943); with tars extracted from atmospheric dusts of several large American cities by Leiter, Shimkin, and Shear; Leiter and Shear (1942), and Kotin, Falk, Mader, and Thomas (1954); with tars obtained from industrial smoke by Clemo et al. (1955); with extracts of exhaust of gasoline and Diesel motors by Kotin, Falk, and Thomas (1954, 1955), Clemo et al. (1955), and Brockbank (1950), and with aromatic polycyclic hydrocarbon-free, aliphatic atmospheric extracts by Kotin and Falk (1955), and Kotin, Falk, and Thomas (1955). The last-mentioned investigators also succeeded in eliciting alveolar tumors in the lungs of C^{57} black mice, which have no tendency toward spontaneous pulmonary tumorigenesis, as well as in accentuating such inherited tendencies in Strain A mice as to time of onset, number of tumors in individual animals, and incidence rate of tumors in the animal population at risk when these animals were exposed to atmosphere-extracted aliphatic hydrocarbons.

In sharp contrast to these experimental observations, indicating a considerable carcinogenic potency of aromatic and aliphatic air pollutants are the inconsistent and poor results obtained with the cutaneous application and inhalation of tobacco and cigarette tar and smoke to mice and rabbits, as well as the equivocal identification and demonstration of any appreciable amounts of any known chemical carcinogen in cigarette smoke. From reviews of the literature by Flory and by Wynder, Graham, and Croninger, it is evident that, with the exception of the results recorded by Roffo, only an occasional cancer was produced in mice or rabbits following a repeated and prolonged application of tobacco tars to the skin or the inhalation of tobacco smoke obtained by various methods of pyrolysis, according to reports published by 1950. This situation has not undergone any fundamental changes in recent years, since the observations of Wynder, Graham, and Croninger, showing a yield of carcinomas of the skin in 44% of the mice painted with tobacco tar, have not

been confirmed as yet by any subsequent investigator using an identical or a similar method of cigarette tar production and repeated and prolonged cutaneous applications of the tar (Passey; Gwynn and Salaman; British Empire Cancer Campaign Report of 1955, and others). The results so far obtained by this experimental approach rather suggest that, in comparison with the carcinogenic potency of coal tar and aromatic and aliphatic air pollutants, tobacco tars are, at most, mildly carcinogenic to the skin of mice. Equally equivocal are the observations recorded during the last few years regarding a carcinogenic action of cigarette smoke on the lungs of mice (Essenberg; Mühlbock; Lorenz, Stewart, Daniel and Nelson) when seen in the light of similar experiments conducted with air pollutants by Kotin and associates, and with coal tar dust by Campbell.

It is noteworthy, moreover, in this connection, that the actual presence of any amount of 3,4-benzpyrene in cigarette smoke has remained a controversial issue, despite numerous investigations by competent workers (Kosak, Swinehart, and Taber; Kuratsune; Lettré and Jahn; Cooper and Lindsey; Cooper, Lindsey, and Waller). It seems that the presence or absence of 3,4-benzpyrene in cigarette smoke may depend upon the method of its production, i. e., the temperature of combustion and the relative oxygen supply as controlled by the number and duration of puffs, and the speed of the air stream through the cigarette smoked in the various smoking machines used. There is serious doubt that the relatively minute amounts of 3,4-benzpyrene claimed to have been demonstrated in cigarette smoke can account for the bulk of the carcinogenic potency allegedly exerted by this medium on the tissues of the lung. Whether the absorption of fluorescent material in cigarette smoke or of anthracen contained in it by the respiratory conduits plays any significant role in the production of bronchiogenic carcinomas is purely speculative (Druckrey and Schmähl; Schmähl, Consbruch, and Druckrey; Schmähl and Schneider).

It has been suggested, moreover, that arsenic content of tobacco, representing an insecticide residue, might play a causal role in the production of lung cancer in cigarette smokers (Doll and Hill; Daff and Kennaway; Oliver; Satterlee; Daff, Doll, and Kennaway; Barksdale). Since the ingestive and cutaneous contact with inorganic arsenicals has resulted in cancer of the skin in man, and while the inhalation of arsenicals apparently has caused cancer of the lung under occupational conditions, not all cigarette tobaccos contain significant amounts of arsenicals. Although "Turkish" tobaccos have as a rule a very low arsenic content, lung cancer rates have risen also in countries where these types of tobacco were predominantly smoked. Nevertheless, it seems to be advisable to keep the arsenic content of tobacco at the lowest possible level, because in most industrialized countries the population has contact with arsenicals from various environmental and dietary sources.

It is, on the other hand, most unlikely that the minute content in tobacco of radioactive material plays any role in any carcinogenic effect claimed for tobacco smoke (Spiers; Spiers and Passey). With a few exceptions in circumscribed areas, similar considerations probably apply to the episodic and permanent increases of background radiation of local and world-wide character related to fall-out of radioactive atomic debris and to activities of the radiochemical-processing industry which have occurred during recent years (Parker; Machta, List, and Hubert; Eisenbud and Harley). This situation, however, may be subject to change, depending upon the type and degree of future developments in radioactive operations (Hueper).

Mention must be made of the presence of a carcinogenic fungicide, 8-oxyquinoline, which appears in the main smoke stream in some tobaccos (Wagner). When implanted into the bladder of mice, it has produced

papillomas and carcinomas (Boyland and Watson). The appearance of uterine cancers in rats following a daily instillation of a spermicidal contraceptive containing this chemical also suggests a carcinogenic action of 8-oxyquinoline (Hoch-Ligeti).

In addition to the potential role of general air pollutants (incomplete combustion products of carbonaceous fuels, oxidation and polymerization products of aliphatic hydrocarbons formed from volatilized gasoline constituents, arsenicals, and radioactive matter) in the production of lung cancer, locally circumscribed contamination of the atmosphere with known carcinogenic materials associated with the release of industrial wastes in the form of dusts, fumes, mists, vapors, and gases of industrial establishments producing or handling carcinogenic substances may possibly create such hazards for persons living or working in the vicinity of such plants. A good illustration of this circumscribed type of industrial air pollution was recently furnished by Bourne and Rushin, who determined the ground-level concentrations of chromium versus leeward distance from a chromate manufacturing plant (Fig. 9).

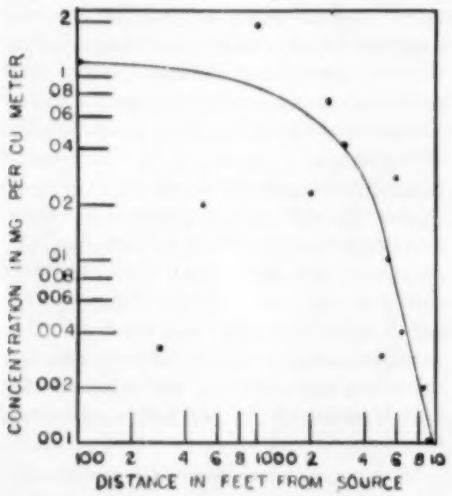


Fig. 9.—Ground-level concentrations of chromium versus leeward distance from source. From Bourne, H. G., and Rushin, W. R.: *Indust. Med.* 19:568-569, 1950; published by the Industrial Medicine Publishing Company, Chicago.

The presence of yellowish and greenish chromium deposits on the ground in the vicinity of chromate plants provides additional evidence of an environmental contamination with carcinogenic matter from such sources. A similar pollution may result from an extensive use of chromates as rust and corrosion inhibitors in automobiles, particularly in communities in which salt is employed for the removal of ice and snow from the streets and where, because of the usage of chromates for protective purposes, the snow may become yellow-colored from spilled chromates (Davis). The large-scale use of barium and zinc chromate paints for the protection of airplanes against corrosion also has provided a source of relative widespread air pollution with these carcinogenic materials through their release into the atmosphere with the exhaust of airplane manufacturing or maintenance plants.

The possibility or actuality of similar local atmospheric pollutions with carcinogenic industrial effluents exists in the neighborhood of smelters handling arsenic-containing ores (copper, zinc, silver), brass foundries, radioactive-ore smelters and refineries, petroleum and coal tar refineries, carbon-black plants, coke ovens, steel plants, gas houses, railroad switchyards, asbestos factories, beryllium plants, and other industrial establishments handling, producing, and shipping carcinogenic materials affecting the respiratory system. While the degree of exposure to these agents for the population living in the environs of such plants will be in general of relatively low order, it is usually a continued one, and therefore should exert a cumulative and additive effect. According to experiences gained in the fields of experimental and occupational carcinogenesis with these and other exogenous carcinogens, it may justly be expected that such exposures in all likelihood may elicit cancerous reactions in some members of the populations at risk.

4. *Occupational Lung Cancers.*—Although some proponents of the cigarette theory of lung cancer have tried to minimize the importance of lung cancer hazards from

TABLE 16.—*Number of Patients with Five or More Years' Exposure in Selected Occupations Among 408 Lung Cancer Cases and 408 Controls* *

Occupation	Lung Cancer Cases	Controls
Total	40	8
Welders	8	2
Crane men and derrick men, exposed to metals	5	0
Firemen, stationary and marine boilers	11	1
Metal miners, copper, lead, and zinc	9	3
Drillers and tool dressers, oil	7	2

* Adapted from L. Breslow (*Dis. Chest* 28: 421-430, 1955).

industrial and industry-related sources in the over-all lung cancer problem (Kreyberg; Kennaway; Doll; McConnell, Gordon, and Jones; Clemmesen and Jensen), there is a substantial body of evidence supporting the view that contact with some such factors is associated with an excessive liability to cancer of the lung among members of a number of large and small occupational groups, some of which sustain exposures to known carcinogens (Breslow, Hoaglin, Rasmussen, and Abrams; Ross; Mancuso; Fulton; Kennaway and Kennaway; Ask-Upmark; Touraine and Bour; Huguenin, Fauvet, and Bourdin; James; Wynder and Graham; Lew; Levin, Kraus, Goldberg, and Gerhardt; Dublin and Vane; Breslow; Hueper; Turner and Grace; Dunner and Hicks; Versluys) (Tables 16, 17, 18, 19, 20, and 21).

In an analysis of lung cancer death rates among policyholders of the Metropolitan Life Insurance Company, Lew noted markedly higher rates among white male industrial policyholders than those for white men in the general population. The reasons for these higher death rates must be sought,

TABLE 17.—*Lung Cancer Death Rates per 1000 Deaths from All Causes for Seven Industrial Groups in Ohio, 5309 Males, 1947* *

Industry	Death Rate
Nonferrous metal	3.22
Transportation	2.91
Rubber and plastics	2.34
Iron and steel	2.18
Mining and quarrying	1.53
Agriculture	0.82
Stone, clay, glass	0.66
Total	1.76

*Adapted from data by Mancuso et al. (*Am. J. Pub. Health* 45: 58-70, 1955).

TABLE 18.—*Incidence of Cancer of Lungs from Insurance Records of the Brotherhood of Locomotive Engineers, Brotherhood of Locomotive Firemen and Enginemen, and Brotherhood of Railroad Trainmen* *

Year	Deaths from Lung Cancer			
	Firemen, per 50,000	Trainmen, per 100,000	Engineers, per 50,000	Total
1942	15	29	16	60
1943	15	28	11	54
1944	16	19	16	51
1945	11	32	10	53
1946	27	51	23	101
1947	29	37	12	78
1948	21	55	15	91
1949	24	63	22	109
1950	21	62	14	97
1951	28	75	28	131
1952	26	75	23	124
Total	233	526	190	949
Increase lung cancer comparing 1942 with 1952	73.33	158.62	43.75	106.67
Increase lung cancer comparing 1942 with 1951	86.67	158.62	75	118.33
Average increase of lung cancer for 10 Yr. 1942 to 1951, inclusive	45.33	71.38	1	38.17

* From data supplied by E. S. Ross in a personal communication.

in his opinion, in some environmental and occupational factors which, according to Lew, are also responsible for the geographical variations in death rates from cancer of the respiratory tract, which are high in most urbanized and industrialized states and low in most agricultural states (Hueper). This observation is in harmony with statistical data of occupational groups showing that farmers have consistently lung cancer death rates at or near the bottom of the scale but skin cancer rates at the top of the list.

TABLE 19.—*Occupational Groups with Excessive Incidence of Lung Cancer*

Occupational Group	Potential Respiratory Carcinogens
Metal workers, welders, metal grinders and polishers, wire makers, tool and die makers, foundry workers, metal molders, lathe workers, boiler scalers	Metal dust, lubricating oil mist
Cigar manufacturers and tobacconists	Tobacco dust, insecticides, soot
Engineers, mechanics, machinists, plumbers, crane operators in smelters, etc.	Metal dust, soot, lubricating oil
Painters, decorators	Metal pigments, coal tar dyes, carbon black, asphalt paints, solvents, vehicles (lacquers, resins, synthetic plastics)
Tar workers, road workers, asphalters, paviors, stokers, patent fuel workers, furnace men, foundry laborers, rollers, etc.	Tar and pitch fumes and dust soot

TABLE 20.—*Cancer of the Lung in English Iron Ore Miners, 1932-1953*

Autopsies	No.	Lung Cancer	Percentage
Iron ore miners	192	17	8.5
Cumberland males over 20 yr.	2378	44	1.85

These epidemiologic data incriminating known and unknown environmental respiratory carcinogens in the production of cancers of the lung provide circumstantial evidence that large occupational population groups comprised of millions of workers have effective contact with potent carcinogens and that, for this reason, industrial agents are not only of potential causal significance in relation to industrial effluents acting upon the general population, but also of distinct importance in connection with specific occupational exposures, operative in special worker groups (Table 22).

TABLE 21.—*Deaths Due to Cancer of the Lung Among Iron Ore Miners and Residents of Minnesota 1950-1954* *

Year	No. of Deaths		Death Rate per 100,000	
	Minnesota Residents	St. Louis-Itasca County Miners	Minnesota Residents	St. Louis-Itasca County Miners
1950	328	5	11.0	37.6
1951	280	4	9.7	30.0
1952	329	12	11.0	90.1
1953	367	8	12.3	60.1
1954	345	6	11.6	60.1

* Adapted from data supplied by J. W. Brower, in a personal communication.

Recent studies of Levin, Kraus, Goldberg, and Gerhardt also showed a positive relation of occupational exposure to iron dust and cancer of the lung. McLaughlin and Harding, who found in autopsies of 85 English foundry workers exposed to iron oxide 13 cases of lung cancer (15.3%), concluded that this evidence strongly suggests that there is an increased incidence of carcinoma of the bronchus in workers with iron and steel.

TABLE 22.—*Specific Worker Groups with Specific Respiratory Cancer Hazards*

Agent	Worker Groups
Arsenic	Manufacturers, handlers, and users of arsenical insecticides; arsenic smelter workers; taxidermists; sheep-dip workers; copper smelter workers
Chromium	Chromate manufacturers, including plant maintenance workers, chrome pigment handlers
Nickel	Nickel-copper matte refinery workers
Iron	Iron ore (hematite) miners (?)
Radioactive substances	Radioactive ore (pitchblende) miners; miners of nonradioactive ores working in radioactive mines
Isopropyl oil	Isopropyl alcohol manufacturers
Coal tar fumes	Coke oven operators; gas-house retort workers
Petroleum oil mists	Paraffin pressers; mule spinners; metal lathe workers and drillers
Asbestos	Miners, textile workers, brake-lining producers

The evidence upon the recognition of these agents as respiratory carcinogens is based on epidemiologic data, clinical and pathological information, and experimental observations (Table 23) (Hueper).

Recent reports from Japan and Great Britain (Yamada, Hirose, and Miyanishi; Case and Lea) have provided strongly suggestive evidence concerning a carcinogenic

TABLE 23.—*Environmental Lung Cancer Score Board*

	Site of Cancer				Population Affected			Type of Evidence			Experimental Evidence	
	Lung	Larynx	Nasal Sinus & Cavity	Nonrespiratory Organs	Occupational	General	Conclusive	Circumstantial	Suggestive	Negative	Lung	Other Organs
Arsenic				Skin	X	?	X					
Asbestos	X				X	?	X				X	
Beryllium	X					?		X			X	Bone
Chromium	X				X	?	X				?	Bone, conn. tissue
Nickel	X		X		X		X					Bone; conn. tissue
Iron	X				X				X			
Radioactive substances	X		X	Skin, Bone, Hemat. tissue Conn. tissue	X		X				X	Bone; conn. tissue; hemat. poretic tissue, etc.
Isopropyl oil *	X	X	X		X		X					
Polycyclic hydrocarbons Coal tar, soot, pitch	X	X		Skin	X	?	X				?	Skin; Conn. tissue; etc.
Petroleum derivatives Oil mist	X	X		Skin	X			X				Skin; Conn. tissue
Gasoline, Diesel engine exhaust	X				X	?		X				Skin
Gasoline epoxides						?				?		Skin

* Isopropyl oil is the crude liquor from which isopropyl alcohol is distilled.

TABLE 24.—*Latent Periods of Environmental Respiratory Cancers, in Months*

Agent	Cancer Site			
	Lung		Nares and Nasal Sinuses	
	Average Latent Period	Range of Latent Period	Average Latent Period	Range of Latent Period
Asbestos	18	15-48	--	--
Chromates	15	5-47	--	--
Nickel	22	6-30	11	3-26
Tar fumes	16	9-23	--	--
Isopropyl oil	--	--	10	8-16
Ionizing radiation	25-35	7-50	25	19-32

effect of 2-chlorodiethyl sulfide (mustard gas) on the lung and larynx when inhaled for occupational reasons or as the result of mustard-gas poisoning during World War I. The established or suspected carcinogenic action of cross-linking and polymerizing chemicals (asbestos, polypropylenes in isopropyl oil, and 2-chlorodiethyl sulfide) places the cancers produced by these agents into the category of "macromolecular" or "polymer cancers" (Hueper). Since many of the recently developed macromolecular polymers used as plastics, resins, adhesives, films, rubbers, textiles, and plasma substitutes have elicited, when parenterally introduced into rats, malignant tumors of various types, it seems to be advisable to study working groups having respiratory contacts with dusts, mists, and fumes of these agents for possible carcinogenic effects of the lungs, larynx, nasal cavity, and paranasal sinuses. Another potential human carcinogen which during coming years may be shown to elicit cancer of the lung, as it has done in the rat, is beryllium or one of its compounds. Such events may be anticipated whenever an adequate latent or preparatory period has elapsed which usually precedes the appearance of occupational cancers of the respira-

Conclusions

1. The irregular epidemiologic pattern presented by the regional distribution and by the rise in frequency of lung cancers in different countries, states, provinces, and communities; the various frequency rates of these tumors in different socioeconomic and occupational population groups; the higher frequency rates of pulmonary cancers among residents of urban and industrialized areas than among those of rural areas; the start of the increase of lung cancer frequency during an era in which cigarette smoking was still uncommon; the demonstration of some eight specific and potent respiratory carcinogens causing lung cancers among members of restricted occupational groups, and, possibly, exerting an identical effect upon members of much larger industrial worker groups having contact with these agents, implicate certain industry-related substances present as occupational and general environmental air pollutants in the causation of lung cancers and in its increased incidence during the last five decades.

2. The data available on the suspected action of these agents are inadequate for making any reliable estimation of the relative quantitative role which they may have played in this respect. However, the epidemiologic, clinical, pathologic, and experimental evidence on hand is adequate for assigning to these agents a considerable part in these matters, since the distribution and growth pattern of industries associated with them exhibit a high degree of similarity to the regional and population distribution pattern of lung cancers and their male-female ratios as related to periods, regions, and population groups.

3. These observations, considerations, and interpretations of a large mass of factual and circumstantial evidence obtained from various sources and collected from different viewpoints do not favor the concept that the great majority of lung cancers, particularly those in men, are caused by excessive cigarette smoking. The epidemiologic evidence concerning this factor, on the other hand, is sufficiently impressive to attribute to cigarette smoking a definite, while less direct or indirect, role in the production and rise in frequency of cancers of the lung. This conclusion is also supported by the fact that cigarette tar proved to be a weak carcinogen to the skin of mice in the hands of most investigators; that cigarette tar, unlike

coal tar, does not elicit cancers of the human skin or lip; that cigarette smoking has no consistently positive statistical relation to cancers of the tongue and oral cavity, although the cigarette smoke has the most intense contact with the mucosa of these tissues, and that serious statistical inconsistencies and irregularities exist concerning regional cigarette consumption and degree of liability to lung cancer among exposed population groups. This assessment of the probable role of cigarette smoking in the lung cancer problem, however, in no way weakens the fact that excessive cigarette smoking is an unhealthy habit for this and other reasons, and therefore should be discouraged.

4. The observation of lung cancer in persons having no appreciable exposure to either the known or the suspected industrial respiratory carcinogens or cigarette smoke suggests that, in all likelihood, other environmental carcinogens of still unknown nature seem to act upon the pulmonary tissue, and that they may enter the human organism by other than the respiratory route.

5. The etiologic panorama of lung cancers has evidently many aspects, most of which have not adequately been explored for arriving at reliable quantitative estimates as to their relative role. The information on some restricted aspects, however, is sufficient for devising and instituting rational and effective preventive measures.

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Neuroblastoma and Related Tumors

MAJOR DANIEL STOWENS (MC), U. S. Army

Since the classic report of Cushing and Wolbach [1] on the apparent maturation of a neuroblastoma to a ganglioneuroma in 1927, the hypothesis that these tumors are essentially different manifestations of the same disease has received general acceptance.[2] The occurrence of tumors containing elements common to both neuroblastoma and ganglioneuroma lends credence to this view. Translation of this schema into practicable terms implies that correlation is possible between the histologic apearance of a tumor and its clinical course; i. e., the more maturation the tumor displays the more benign its course is likely to be. This approach leads logically to the attempt to subdivide tumors arising from the sympathetic nervous system into various categories according to their cytologic characteristics. However, the bases for differentiation are largely subjective and the criteria by which the tumors in reported series are assigned to one category or another are not consistent. This gives rise to difficulties in attempting a comparison of the results in one series with those of another.[3-6]

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Armed Forces Institute of Pathology, Washington, D. C.

TABLE 1.—*Tumors of Sympathetic Nervous System Studied*

Classification	Total Cases	Follow-Up No.	Completed %
Neuroblastoma	110	105	96
Ganglioneuroma	109	85	78
Ganglioneuroblastoma	17	16	94
Total	236	206	87

In an effort to clarify the interrelations of the tumors of the sympathetic nervous system, a study of a large series of cases was undertaken. The material was drawn from the files of the Armed Forces Institute of Pathology. The classification and number of tumors of each type are presented in Table 1.

Methods

The microscopic slides on every case with the diagnosis of a tumor arising from the sympathetic nervous system were examined. According to histologic and cytologic criteria the tumors were

TABLE 2.—*Distribution of Tumors According to Primary Site and Age*

Age Group, Yr.	Neuroblastoma				Ganglioneuroma				Ganglioneuroblastoma			
	Adrenal	Retroperitoneum	Mediastinum	Other*	Adrenal	Retroperitoneum	Mediastinum	Other†	Adrenal	Retroperitoneum	Mediastinum	Other‡
0-1	24	15	4	5		1	1			2	1	1
2-4	12	7	3	5		2	1		2	1	1	
5-9	3	2	1	1		2	2			1		
10-14	1	1	1	2								
15-19		3	3			2	7			1		
20-29	2	10	2	1	8	11	8			3		
30-49	1	1		1	7	2	11	2		1		
50+			1		19	4	4	2				1
Unknown					1	1	1	2				
Total	43	39	14	14	33	20	41	15	2	11	2	2
			110				109				17	

* Neck (5), maxilla, humerus, ext. ear, lung, temporal region, mandible, shoulder, unknown, 2.
† Neck (6), prostate, chest wall (2), thigh, sacral nerve, elbow, cranial nerve, appendix, bladder.
‡ Neck (2).

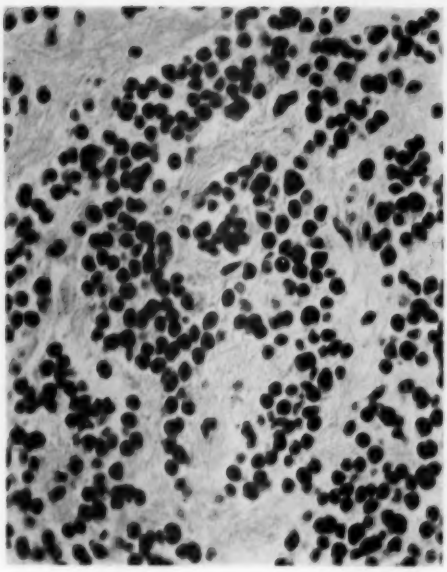
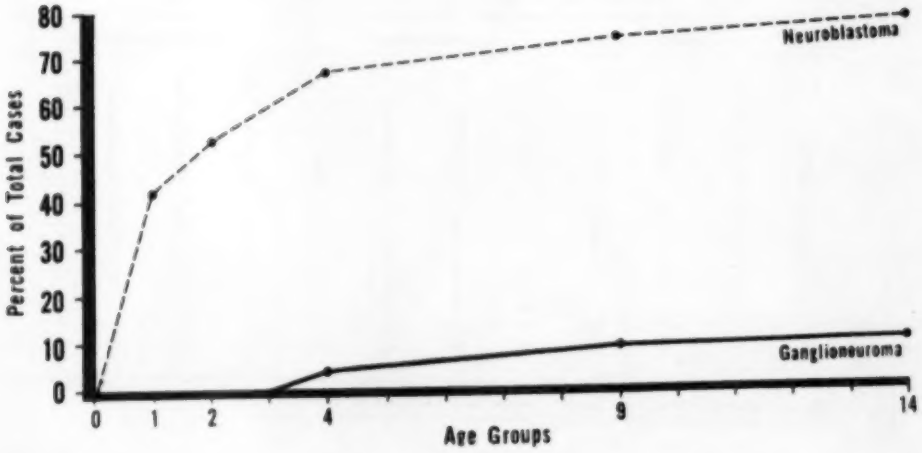


Fig. 1.—Neuroblastoma. The predominant cell type is the neuroblast. AFIP Acc. 719003. Hematoxylin-eosin stain; reduced to 80% of mag. × 440.

Percentages of Total Cases, Cumulative, by Age Groups

AGE	NEUROBLASTOMA	GANGLIONEUROMA
1 Year	42	0
2 Year	52	0
4 Year	67	4
9 Year	74	9
14 Years	78	10



Graph 1

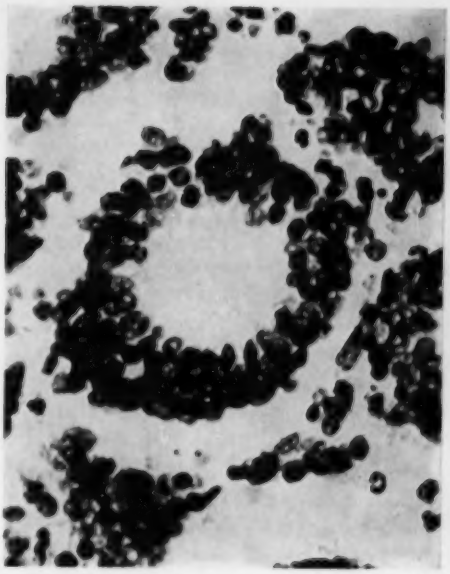
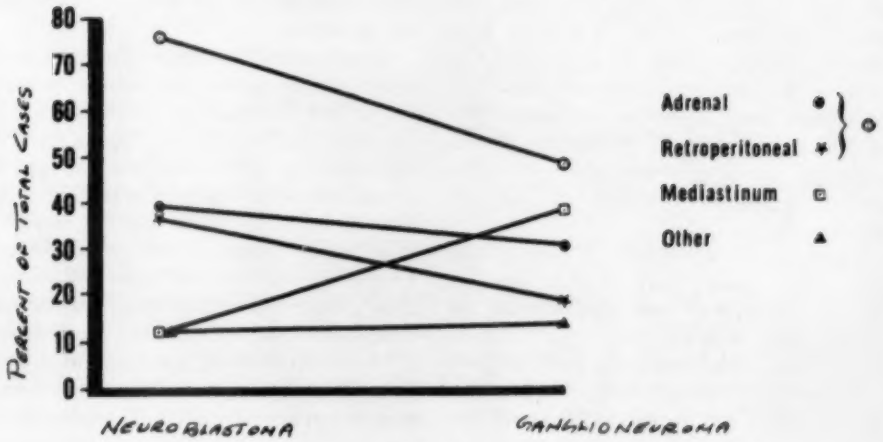


Fig. 2.—Neuroblastoma. Rosette. These are found in about 15% of neuroblastomas. AFIP Acc. 489139. Hematoxylin-eosin stain; reduced to 80% of mag. × 650.

Primary Site of Lesions, Percentages

	NEUROBLASTOMA		GANGLIONEUROMA	
Adrenal	39	76	30	48
Retroperitoneal	37		18	
Mediastinum	12		38	
Other	12		14	



Graph 2

divided into three groups: neuroblastoma, ganglioneuroblastoma, and ganglioneuroma. Only after this was accomplished were the clinical records consulted and clinicopathologic correlations attempted. In all instances the preparations were stained with hematoxylin and eosin. Various special stains (i. e., Masson trichrome stain, Bodian silver technique, phosphotungstic acid-hematoxylin) were found to add little information. The distribution of tumors of the various types according to age and primary site is given in Table 2.

Criteria of Classification

Neuroblastoma. — Gross Characteristics: The tumors varied in size, the primary rarely being almost microscopic, but usually clinically discernible. Ninety per cent of the lesions in this series had no capsule. Typically, the tumor was soft and sometimes friable. Hemorrhage and necrosis were evident grossly in 55%. The color was most frequently gray but appeared darker in those tumors with considerable hemorrhage.

Histologic Characteristics: The predominant cell type is the neuroblast (Fig. 1). This cell is small, 10μ to 15μ in diameter, with a large nucleus and little cytoplasm. The chromatin material is dense and the intranuclear details obscure. Variations are common, and tendencies toward definitive differentiation may be seen; i. e., the cytoplasm may form fibrillary structures quite similar to neurofibrils. The tumors have a varied architectural pattern, but most consist of sheets of cells spreading uninterruptedly in all directions. There is usually little stroma. Rosettes (Fig. 2) were found in 15% of the tumors. In tumors which had undergone some degree of necrosis (30% of this series) the cells nearest the fibrous septa and vessels were best preserved. This tended to give such tumors an organoid appearance.

Ganglioneuroma.—Gross Characteristics: The tumors vary in size but are always encapsulated and firm and on cut surface have a pearly-gray color. Calcification was evident macroscopically in 23%.

Histologic Characteristics: The mature ganglion cell is the hallmark of this tumor (Fig. 3). Such cells occur in varying numbers and may be scattered singly throughout the tumor or arranged in clumps. The stroma is abundant and dense and may contain stainable neurofibrils, as well as collagenous fibers. The stroma may show considerable variation in pattern, and in this series arrangements reminiscent of neurofibroma and neurilemmoma were seen. It seems probable that such tumors may arise from sympathetic, as well as other peripheral nerves.

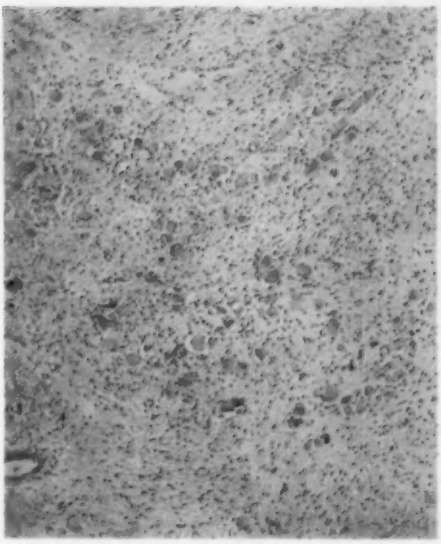


Fig. 3.—Ganglioneuroma. The mature ganglion cell is the hallmark of this tumor. AFIP Acc. 134672. Hematoxylin-eosin stain; reduced to 80% of mag. × 175.

Ganglioneuroblastoma. — Generalizations about tumors of this type cannot be made, for in both histologic and gross characteristics they combine, in varying degree, the characteristics of the neuroblastoma and the ganglioneuroma. The cells in any one tumor may all be of the same type or may vary from mature ganglion cells to neuroblasts. Frequently the predominant cell is one clearly related to a ganglion cell but showing eccentricities of the nucleus, great density of chromatin material, and diminution in the amount of cytoplasm (Fig. 4). Several tumors appeared to be predominantly

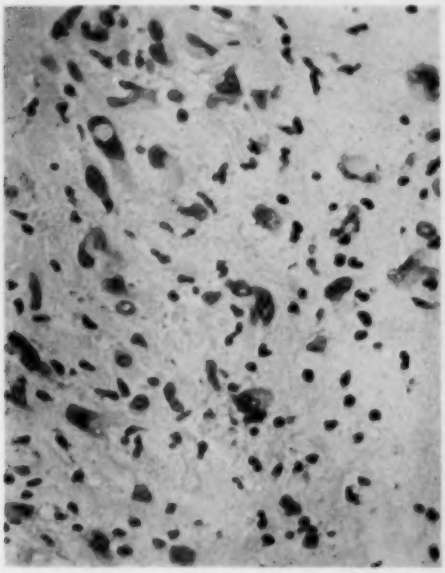


Fig. 4.—Ganglioneuroblastoma. The predominant cell is the abnormal ganglion cell. AFIP Acc. 315261. Hematoxylin-eosin stain; reduced to 80% of mag. × 440.

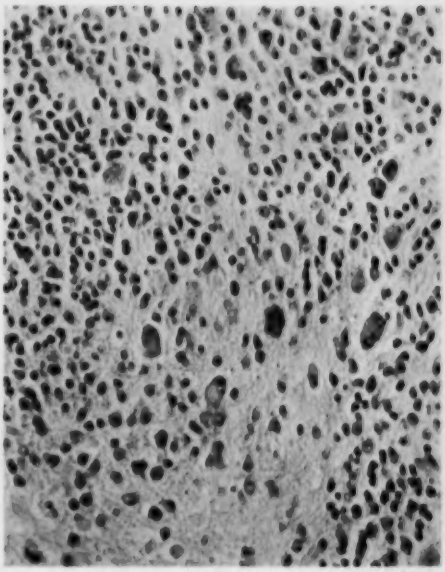


Fig. 5.—Ganglioneuroblastoma. A mixture of abnormal ganglion cells and neuroblasts is seen. AFIP Acc. 522785. Hematoxylin-eosin stain; reduced to 80% of mag. × 260.

ganglioneuroma in one portion and predominantly neuroblastoma in another, although more frequently the two types of cells were intermingled (Fig. 5). An attempt to differentiate tumors of this group into those showing more and less differentiation proved fruitless because of the lack of clear-cut distinctions, and, later, because no correlation with clinical course could be found in the subgroups.

Symptomatology

Neuroblastoma and Ganglioneuroblastoma.—The symptoms manifested by patients with neuroblastoma and ganglioneuroblastoma were essentially alike. They were, in decreasing order of frequency, according to systems, gastrointestinal (including palpable abdominal mass), 49%; respiratory, 20%; skeletomuscular, 20%; hemic (including weakness due to anemia, malaise, etc.), 20%; skin (palpable mass), 8%; urinary (hematuria or flank pain), 2%. Only 8% of the patients had no symptoms, and the tumor was discovered on routine physical or x-ray examination.

Ganglioneuroma.—Of the patients with ganglioneuroma, only 40% had symptoms referable to the tumor. Almost all of these were respiratory and occurred in patients with mediastinal neoplasms. In general, the abdominal ganglioneuromas were found either on routine physical or on roentgen examination, or incidentally at autopsy. The only other symptoms manifested by the patients in this group were of a local nature, resulting from pressure caused by the tumor.

Clinical Course

Ganglioneuroma.—All of the tumors in this group were benign. There were no metastases, and only one death was attributable to the tumor itself. This occurred in a patient 54 years of age. The tumor arose from one of the cranial nerves at the base of the skull and proved to be inexcisable. The patient died of the mechanical side-effects of pressure. Two patients had von

Recklinghausen's disease, and several of their tumors proved to be ganglioneuromas.

Neuroblastoma.—Of the 105 patients on whom complete information is available, 14 (13%) have survived for 18 months or longer. The over-all survival rate is misleading, however, for if the series is divided according to the age of the patient, it is seen that all of the survivors were less than 4 years of age and all but one were in the first year of life. The precentage of survivors in the first year is 29%; in the two- to four-year age range, 4%, and in the older age group, 0 (Table 3). The average time of survival in those patients who succumbed to the disease was 11 months. Seventy-one per cent of the patients died within the first year and only 3% of those who eventually died of the disease survived for more than three years. Metastases occurred to the following sites, in decreasing order of frequency: liver, 65%; regional lymphatics, 60%; lungs, 60%; skeleton (not including bone marrow or skull), 50%; gastrointestinal tract, 43%; adrenal, 31%; bone marrow, 23%; skull, 23%; kidney, 19%; orbit, 17%; lower genitourinary tract, 13%. Only nine patients (8%) had no recorded metastases, and four of these were stillborn or newborn infants.

TABLE 3.—*Distribution of Patients* with Neuroblastoma and Ganglioneuroblastoma According to Age, Number, and Per Cent of Survivors*

Age, Yr.	Completed Cases	Survivors† No.	Survivors† %
Neuroblastoma			
0 — 1	45	13	29 (33)‡
2 — 4	27	1	4
5 — 9	7	0	0
10 — 14	4	0	0
15 — 19	5	0	0
20 — 49	16	0	0
50 +	1	0	0
Total	105	14	13
Ganglioneuroblastoma			
0 — 1	4	2	50
2 — 4	5	2	40
5 — 19	2	0	0
20 +	5	1	20
Total	16	5‖	31

* Patients with complete data.

† Survivors: Patients living at least 18 months without evidence of recurrence and/or metastases.

‡ Five of the tumors occurred in newborn or stillborn infants dying of causes other than tumor. If these are eliminated from the total number, the percentage of survival is as indicated in parentheses.

‖ Three additional patients have survived 18 months or longer but have had recurrence and spread of their disease. The values shown are projected to exclude them.

Ganglioneuroblastoma.—Complete data are available on 16 of the 17 patients with tumors of this type (Table 3). At the last report, eight of the patients were alive (50%) but three of the survivors had evidence of metastasis. The projected survival rate is, therefore, 31%. The course of the disease in the fatal cases was slower than in the fatal cases of neuroblastoma, 22 months on the average elapsing between the time of diagnosis and death. The metastases generally showed the same distribution as those of the neuroblastoma.

Comment

Eight of the fourteen surviving patients with neuroblastoma had essentially identical histories. All were under one year of age and were asymptomatic. Abdominal masses were palpated on routine physical examination, and operation was performed. In all cases the tumor was described as retroperitoneal, arising from a point between the kidney and the adrenal but not involving either of those organs. All tumors were believed to be encapsulated, and in five instances a fibrous connection to the pelvis of the kidney was described. Total removal was easily accomplished, and the patients have remained free of disease for periods of from 26 months to 8 years. The patient surviving the longest had a tumor of the external auditory canal. He has been followed for 18 years, with no evidence of recurrence.

Two of the patients less than one year of age had posterior mediastinal tumors, both masses being described as encapsulated. One of these tumors showed direct anatomic continuity with a sympathetic nerve; the other did not. Two patients, both asymptomatic, had tumors arising within the adrenal, producing a palpable abdominal mass. One of these patients was believed to have hepatic metastases at the time of operation and was given postoperative roentgen therapy. He

is alive and well, 26 months later. The remaining survivor was 10 months of age when a mass was palpated in the scalp. This was excised and a diagnosis of metastatic neuroblastoma was made. An exploratory laparotomy was performed, and a tumor was found in the left adrenal. She has been followed 30 months, without a recurrence of the disease.

It was noted, in reviewing the records of these patients, that 10 of the 14 survivors had a tumor described as encapsulated both by the surgeon and by the pathologist. In the fatal cases only two of the primary tumors were so described. The attachment of the tumor to the pelvis of the kidney noted in five of these patients was not described in any of the fatal cases. It is of interest that almost all these patients were asymptomatic and that the disease was discovered during the course of an examination for other purposes. All the patients who failed to survive showed definite symptoms or signs.

A review of the data from the patients with ganglioneuroblastomas showed that the preoperative clinical course and the gross or microscopic appearance of the tumor were essentially the same in all tumors whether benign or fatal. An attempt to correlate the histologic characteristics of the tumor with the clinical course of the patient proved a total failure. Some of the survivors had tumors which appeared to be highly malignant, whereas some who succumbed had tumors which appeared to be relatively benign.

In order to determine the relation of the neuroblastoma to the ganglioneuroma, the data were analyzed from two additional aspects: the site of occurrence of the primary tumor and the age of the patient. These data are presented in Graphs 1 and 2. In Graph 1, constructed from data in Table 3, the values represent the cumulative percentages of tumors occurring at the indicated ages. It is seen that neuroblastoma is primarily a disease of early childhood (approximately 70% of the cases occur in the first four years of life), whereas ganglioneuroma is predominantly a disease of adult life. No ganglioneuroma was found in patients less than 4 years of age. Were the ganglioneuroma and neuroblastoma different by virtue of differences in rate of growth, it would be necessary to postulate a difference in such growth rates of 81 years (according to the ages of the patients in this series).

Were the two tumors merely different manifestations of the same disease, they should occur at various sites with the same frequency. Graph 2 presents the actual percentages of occurrence of the neuroblastoma and the ganglioneuroma at specific sites. Although there is considerable overlapping, there is also a significant difference in the values for the rate of occurrence at the commonest sites.

From the differences in anatomic characteristics, clinical behavior, and the ages and sites at which the tumors occur, it is believed that the neuroblastoma and ganglioneuroma are essentially different diseases of the same system, rather than different manifestations of the same disease.

The neuroblastomas as a group present a paradox. It is certain that some of these tumors are congenital in origin. Histologically identical tumors occur at any age, however, and if the postulate of the congenital origin of neuroblastoma is strictly maintained, it is necessary to assume that the tumors lie dormant for prolonged periods of time or have vastly different growth rates. Neither of these assumptions seems probable.

A second difference in the neuroblastomas occurring in the various age groups was discovered when the site of occurrence of the primary tumor was considered in relation to the age of the patient. Whereas the adrenal was the site of the neuroblastoma in 51% of the patients under the age of 3 years, it was the primary site in 35% of the patients in the 3- to 14-year age group and in only 13% of the adult patients.

It would appear from these data that the tumors classified on histologic grounds as neuroblastoma actually comprise two groups.

The first is a tumor of congenital origin and probably results from the disruption of the normal embryogenesis of the sympathetic nervous system. This congenital tumor occurs within the first few years of life, is amenable to surgical treatment in about 30% of the patients, and may even undergo spontaneous regression. The second type of tumor probably results from malignant degeneration of fully mature elements of the sympathetic nervous system, and would more properly be designated neuroblastic sarcoma. The frequency of occurrence of this tumor is the same throughout the life span for all age groups. It is always fatal.

The problem is somewhat clouded by the occurrence of the tumors designated ganglioneuroblastoma. Histologically these neoplasms bridge the gap between the neuroblastoma and the normal sympathetic nervous system, but the bridge is merely apparent. Theoretically, it is possible that tumors incorporating features common to normal tissue and neuroblastoma may arise in two ways. First, in the course of malignant degeneration of tissues of the sympathetic nervous system some resemblance to the parent tissue may be retained, or, second, in the case of the congenital neuroblastoma, the tissue may attempt definitive differentiation despite its disturbed development. In order to differentiate this process from the malignant degeneration occurring in mature tissue, it is suggested that such partial differentiation be called "pseudodifferential embryogenesis."

The difficulty in establishing clinicopathologic correlation for ganglioneuroblastomas is believed to arise from the dual origin of such tumors and the differences in the behavior of the parent tumor type. Although a prognosis cannot be made in any individual case, as a group these tumors are less malignant than neuroblastomas.

This theoretical schema of the origin of tumors of the sympathetic nervous system is presented in Figure 6. The "benign neural crest rests" indicated in the chart are composed of cells which apparently are neuroblasts. They occur primarily in the dermis; and, although they can usually be identified by the greater amount of stroma, their organized appearance, and the absence of signs of active growth, they may be confused with metastatic lesions Most of these lesions have been seen in children and have been removed for cosmetic or prophylactic reasons. One has been seen in a woman 34 years of age.

The question of whether or not benign ganglioneuromas ever undergo malignant

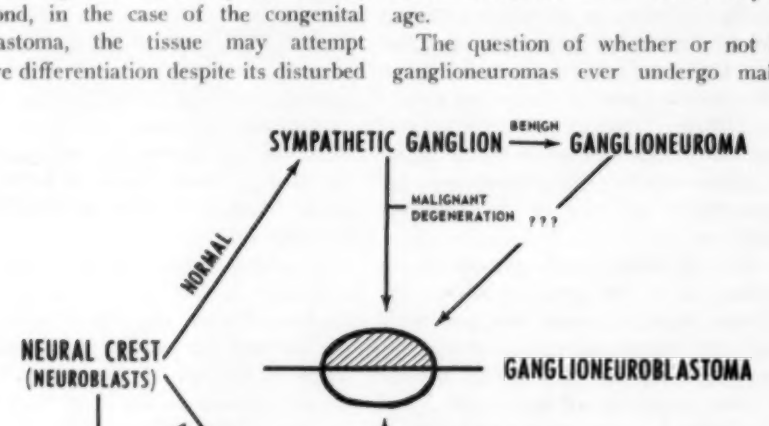


Fig. 6.—Schema for origins of tumors of the sympathetic nervous system.

degeneration must remain unanswered. I can only state that in my experience I have never seen this occur.

That there is any difference between "malignant degeneration" and "pseudodifferential embryogenesis" cannot be proved. It is true that they may be the same process with only apparent differences arising because of the stage of development of the affected tissues. Yet, because of the real differences in the biologic behavior of the resultant tumors, a differentiation appears justifiable, at least at the present stage of knowledge.

Conclusions

The tumors of the sympathetic nervous system may be divided into four groups: congenital neuroblastoma, neuroblastic sarcoma, ganglioneuroblastoma, and ganglioneuroma. The congenital neuroblastoma and the neuroblastic sarcoma are indistinguishable anatomically but must be separated because of differences in their biologic behavior. The congenital neuroblastoma probably always occurs within the first six years of life and is fatal in 70% of the cases. The neuroblastic sarcoma occurs with random frequency throughout life and is invariably fatal. The ganglioneuroma is a benign tumor of mature tissue. It occurs mainly in the adult age group. The ganglioneuroblastoma is a tumor which combines features of both normal ganglionic tissue and neuroblastoma. It is essentially only a histologic designation rather than a distinct entity, for it is probably a manifestation of either a lower degree of malignant degeneration of a neurogenic sarcoma or a partial degree of differentiation of a neuroblastoma. Because of the difference in prognosis in this group (69% are fatal) and that of the congenital neuroblastoma and neuroblastic sarcoma, the ganglioneuroblastoma is treated as a separate entity.

Miss Ethel Hicks gave invaluable assistance in the compilation and statistical analysis of the data on which this report is based.

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Arteriosclerosis in the Baboon

Naturally Occurring Lesions in the Aorta and Coronary and Iliac Arteries

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Arteriosclerosis, often associated with vascular lipid infiltration, is common in many mammalian species. This degenerative process has been rarely described, however, in subhuman primates, either as a naturally occurring disease or as a lesion produced by experimental means.

Fox [1] studied cardiovascular specimens from 796 primates. No arteriosclerotic lesions were observed in higher apes (chimpanzees, orangs, and gibbons) or in lemurs, and the disease was present in only 8 of 693 New and Old World monkeys. In these eight monkeys, superficial atheromatous patches in the sinuses of Valsalva and in the thoracic aorta were found. The abdominal aorta was involved rarely. Earlier lesions consisted of thickening of subendothelial fibrils and inconspicuous elastic fibers with lipid droplets between them. In more advanced lesions, the thickened intima was hyalinized and contained a few elastic fibrils and calcific granules. The internal elastic membrane was split and frayed. A single baboon examined by Fox [1] had an atheromatous protrusion at the right coronary arterial orifice.

In an extensive study of a male gorilla, Steiner [2] encountered arteriosclerosis of myocardial, cerebral, and celiac arteries and of the aorta. The smaller vessels were thickened by hyaline fibrosis, which was associated with degeneration of the internal elastic membrane. Intimal lesions of the aorta, not visible grossly, were chiefly fibrous and did not contain lipid.

Accounts of primate arteriosclerosis induced experimentally are as few as those describing naturally occurring vascular lesions. Kawamura [3] failed to find arteriosclerosis in rhesus monkeys fed cholesterol for as long as 10 months. Although Sperry and co-workers [4] did induce mild hypercholesteremia in rhesus monkeys by cholesterol feeding or by thyroidectomy, arterial lesions were not observed in these animals. Hueper [5] was also unsuccessful in producing arteriosclerosis in young rhesus monkeys by feeding cholesterol.

Significant studies in the pathogenesis of arteriosclerosis were reported by Rinehart and Greenberg,[6,7] who found arteriosclerosis of the aorta and other arteries of rhesus monkeys subjected to prolonged pyridoxine deficiency. The lesions were characterized by deposits of mucinous material in the intima and later by cellular proliferation and formation of collagenous and elastic fibers in the intimal layer. Lipid infiltration of the intima was not a prominent feature of the disease and was observed only in the deeper layers of older sclerotic intimal lesions. The lesions induced by pyrodoxine deficiency closely resembled those of human arteriosclerosis and were quite similar to lesions observed in a number of avian and mammalian species.

The most recent contribution to the experimental study of primate arteriosclerosis is that of Mann and co-workers,[8] who described arterial disease in cebus monkeys fed diets high in cholesterol and low in sulfur amino acids. Vascular lesions were observed throughout the aorta, but were minimal in the coronary arteries. The ar-

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terial disease was characterized mainly by intimal collections of foam cells containing free and esterified cholesterol and neutral fat. Increased amounts of ground substance or metachromasia were not observed.

The present report deals with naturally occurring arteriosclerosis in another primate species, the Doguera baboon (Papio anubis). The lesions in the aorta and coronary and iliac arteries bear a close resemblance to degenerative arterial lesions of other mammalian species, including man. Lipid infiltration of the intima lesions, like that of other species, is minimal and is clearly a secondary phenomenon.

Materials and Methods

This report is based upon the examination of the hearts, aortas, and iliac arteries of two male Doguera baboons, each about 20 years of age. These animals had lived continuously in the San Diego Zoo and had been fed a diet of seasonal vegetables and fruits (lettuce, carrots, potatoes, oranges, bananas, apples, grapes, watermelon) and bread.

The tissues were submitted for examination following fixation in 10% formaldehyde solution U. S. P. After gross inspection, blocks of tissue were removed from the ventricular walls, the major coronary arteries, and several sites in the thoracic and abdominal aortas and common iliac arteries.

Contiguous frozen sections from each block were stained with (1) Sudan IV and hematoxylin and (2) Nile blue (Nile blue sulfate). An unstained frozen section was used for examination with polarized light. These blocks were then embedded in paraffin, and contiguous sections were treated with hematoxylin and eosin stain, Laidlaw's connective tissue stain, the Weigert-Van Gieson stain, and colloidal iron-Prussian blue (Ferric Ferrocyanide) stain.

Gross Description

Heart.—No abnormalities of the pericardial, myocardial, or endocardial layers were observed in the heart of either animal. The major coronary arteries had thin, pliable walls, and gross evidence of disease was not observed. The valvular cusps were thin and filmy, and lipid or fibrous lesions were absent.

Thoracic Aorta. — Mild, diffuse intimal thickening, appearing gray and fibrous, was present on the convex surface at the apex of the thoracic arch of one animal. Near the site of the closed ductus arteriosus and below the apex of the arch, in the second animal, were several 0.5 mm. white, fibrous plaques, usually lying adjacent to vascular openings; slightly larger, round, fibrous plaques lay near the opening of the celiac axis. In the first animal there were multiple slightly elevated, linear yellow streaks in the intima of the thoracic arota. These plaques lay below the openings of the majority of the first seven intercostal arteries and averaged 0.5×3 mm. in diameter.

Abdominal Aorta.—In the second animal there were numerous white, fibrous plaques in the aortic intima. These averaged 1 to 2 mm. in diameter and appeared as coalescing groups or elongated streaks, involving much of the circumference of the abdominal aortic wall.

Common Iliac Arteries.—The second animal had larger and more numerous similar plaques in the common iliac arteries. The majority were pale white and fibrous, but several were distinctly lemon yellow.

Microscopic Description

Myocardium.—The left ventricular wall of both baboons showed extensive disease. Lymphocytes and large mononuclear cells infiltrated the pericardial fat, and there was early minimal fibroblastic proliferation. The lumen of one small pericardial coronary artery contained a hyaline thrombus, which was covered on one surface by hypertrophied endothelial cells. The wall of this vessel was not otherwise altered. Extensive fibrosis replaced large segments of the myocardial layer, especially near the pericardium and endocardium. The connective tissue was loosely arranged, vascular, and mildly infiltrated with lymphocytes (Fig. 1). Atrophic myocardial fibers were isolated by the connective tissue. The remaining myocardial fibers and their nuclei showed pronounced hypertrophy. Focal degenera-

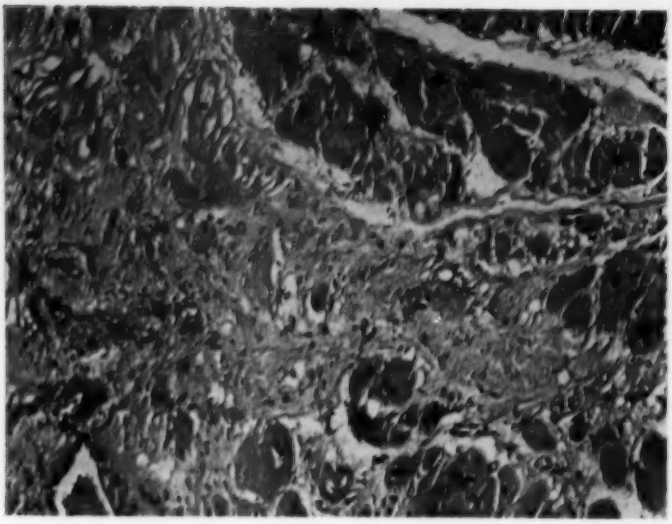

Fig. 1.—Left ventricular myocardium. Fibrous tissue infiltrated with lymphocytes replacing myocardial fibers. The residual fibers are hypertrophied. Hematoxylin and eosin stain; reduced to 90% of mag. × 100.

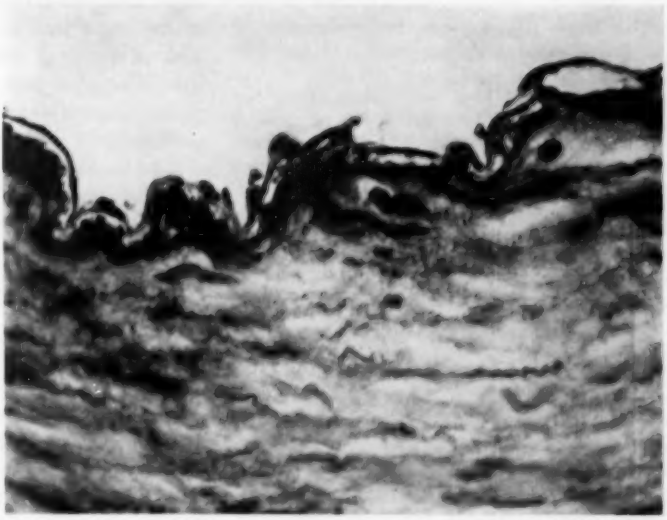

Fig. 2.—Major coronary artery. The internal elastic membrane is severely fragmented. The adjacent dark substance is mucopolysaccharide. Colloidal iron-Prussian blue stain; reduced to 90% of mag. × 600.

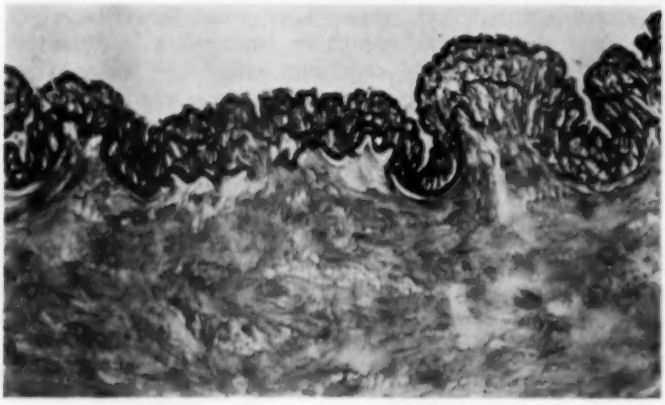

Fig. 3.—Major coronary artery. The thickened intima contains both fragmented and regenerated elastic fibers. Weigert-Van Gieson stain; reduced to 90% of mag. × 200.

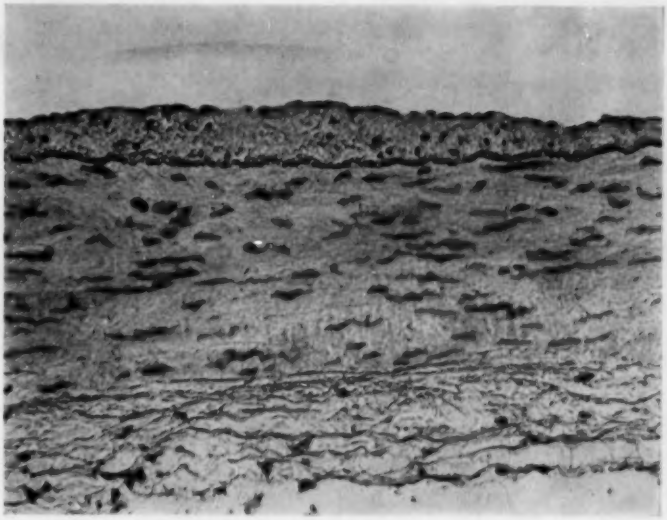

Fig. 4.—Major coronary artery. The intima consists of amorphous substance. Note the granular and broken internal elastic membrane. No lipid is present. Sudan IV-hematoxylin stain; reduced to 90% of mag. × 200.

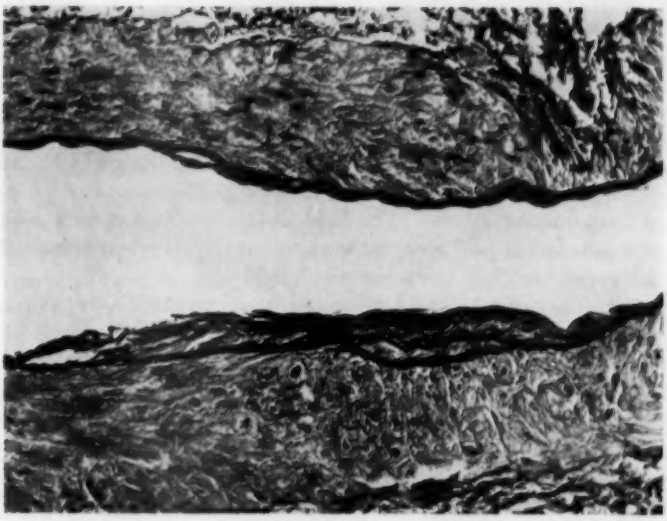

Fig. 5.—Major coronary artery. The intimal plaque contains many coarse elastic fibers. Weigert-Van Gieson stain; reduced to 90% of mag. × 200.

tion of myocardial fibers; infiltration by lymphocytes, polymorphonuclear leukocytes, and fibroblasts, and perivascular fibrosis were observed in much of the myocardial layer. Inflammatory and denegerative lesions were absent in the wall of the right ventricle. No abnormalities of the small coronary arterial or venous vessels were noted.

Coronary Arteries.—In normal coronary arteries, the endothelium lay close to the wavy internal elastic membrane, which was covered on both surfaces by a thin layer of mucopolysaccharide substance. The earliest evidence of disease appeared as fragmented segments of the internal elastic membrane, which were surrounded by increased accumulations of mucoid material. These fragmented segments were swollen, granular, and less distinctly refractile than the normal internal elastic membrane (Fig. 2). Some fragments had been replaced and redupli-

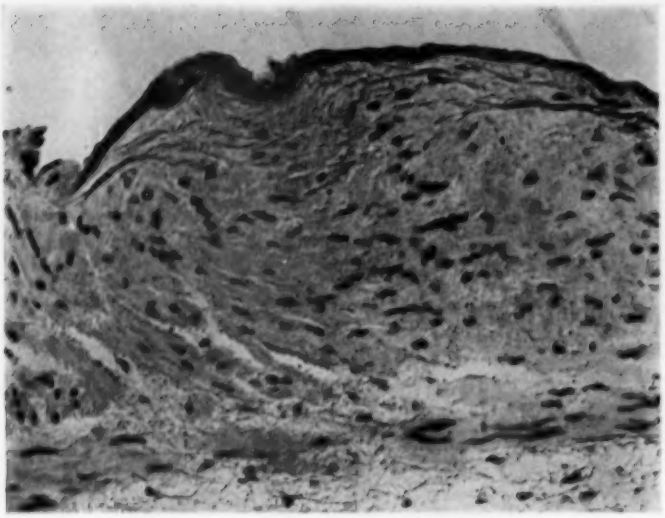

Fig. 6.—Major coronary artery. The intima is not widened, but coalescing lipid particles infiltrate the subendothelial layer near the internal elastic membrane. Sudan IV-hematoxylin stain; reduced to 90% of mag. × 200.

cated by one or more newly formed elastic layers (Fig. 3).

As the coronary disease advanced, eccentric or concentric thickening of the intima occurred. The thickened intima consisted of granular, amorphous eosinophilic substance, containing a few cells resembling endothelium and often having abundant clear cytoplasm (Fig. 4). In some areas of the thickened intima the cells were smaller and had smaller compact nuclei. The cellular arrangement was either vertical or parallel to the endothelial vascular lining. The thickened intima contained delicate elastic, reticulum, and collagenous and mucopolysaccharide fibers, which appeared first immediately adjacent to the intimal cells and presumably were derived from their cytoplasm. As intimal thickening progressed, the fibrils became more abundant and coarser (Fig. 5). The elastic tissue fibers assumed an undulating pattern that resembled closely the original internal elastic membrane. The newly formed, regenerated elastic fibers also showed points of fragmentation.

In the mildly thickened intima of a major coronary artery of one animal no lipid material was demonstrable (Fig. 4). Where intimal thickening was more pronounced, finely divided lipid material, staining with Sudan IV, was present in small amounts scattered in the intercellular substance. In some areas fine lipid droplets were concentrated in and about the fragmented segments of the internal elastic membrane and in the adjacent mucopolysaccharide material (Fig. 6). The lipid stained pale violet with Nile blue, but none was refractile when examined with polarized light.

Thoracic Aorta.—Segments of the intima were mildly thickened and consisted of eosinophilic fibrils and scattered, irregularly arranged cells resembling endothelial cells or fibroblasts. Reticulum, collagenous, elastic, and mucopolysaccharide fibers in the mildly thickened intima were extremely delicate and were most numerous next to the intima cells. Very small amounts of finely divided lipid substance were found in the slightly thickened intima segments, and this lipid was distributed along the wavy elastic fibers in the intima. However, in some segments of the thoracic aorta, where no intimal thickening was present, similar lipid deposits were seen along the superficial elastic fibers adjacent to the endothelium. In areas where intimal thickening was more pronounced, no lipid was present in the intimal layer, but a few intercellular droplets

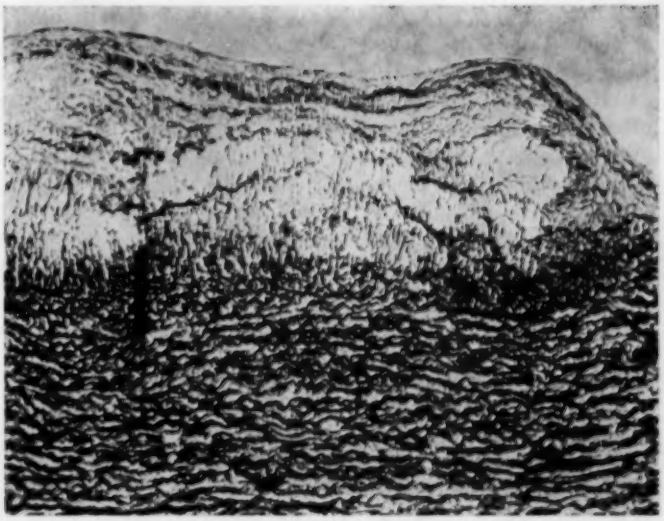

Fig. 7.—Lower thoracic aorta. The intimal plaque consists of mucoid connective tissue containing elastic fibrils. Weigert-Van Gieson stain; reduced to 90% of mag. × 200.

were observed in the media beneath. These early deposits of lipid stained dark violet with Nile blue, but none appeared refractile with polarized light.

Other thoracic aorta lesions were more extensive and involved at least half the circumference of the thoracic aorta. The thickest portions of the intima formed large plaques, composed of connective tissue fibers and cells (Fig. 7). As a rule, these plaques showed distinct layering. The central portions of the plaques tended to be loosely arranged, whereas the superficial and deep parts were more compact. The superficial layers usually displayed an irregular arrangement of the cells and fibers, whereas in the deeper segments the cells and fibers were arranged vertically.

These intimal plaques contained slender reticulum and collagenous fibers, and in some plaques mucopolysaccharide fibers were somewhat more numerous. All this fibrillary substance tended to be concentrated about the intimal connective tissue

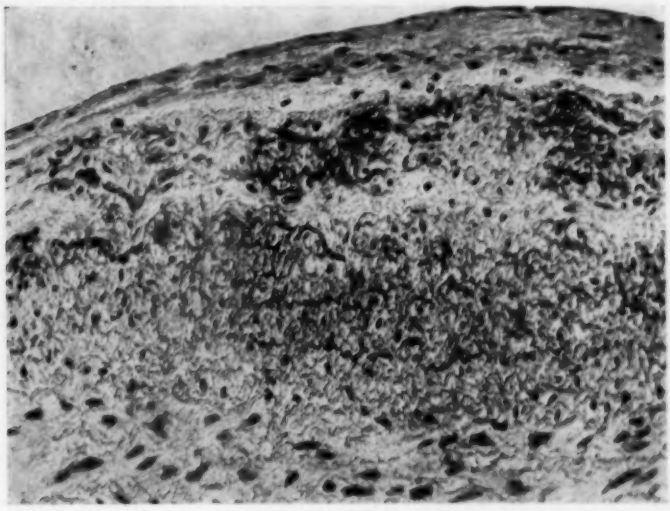

Fig. 8.—Lower thoracic aorta. Lipid infiltrates the deeper portions of the intima. Fine lipid droplets are applied to the intimal elastic fibers. Sudan IV-hematoxylin stain; reduced to 90% of mag. × 200.

cells. Usually the intimal plaques contained abundant elastic tissue, which appeared as undulating bundles lying parallel to the endothelial surface. These elastic fibers were often more numerous in the deeper and more compact segments of the intimal plaques. Although resembling normal wavy elastic fibers, these newly formed fibers consisted of bundles of delicate elastic fibrils and were not refractile.

The larger plaques contained moderate amounts of lipid material, appearing most abundantly in the deeper, more compact segments of the plaques, and for the most part this lipid was closely applied as fine droplets to the elastic fibers (Fig. 8). In the superficial portions of the plaques, where the cells were large and had greater amounts of cytoplasm, larger, coalescing lipid droplets filled the cytoplasm (Fig. 9). This lipid material, as a rule, stained blue to violet with Nile blue. In the midportion of one plaque, groups of refractile, elongated, needleshaped crystals were seen with polarized light (Fig.

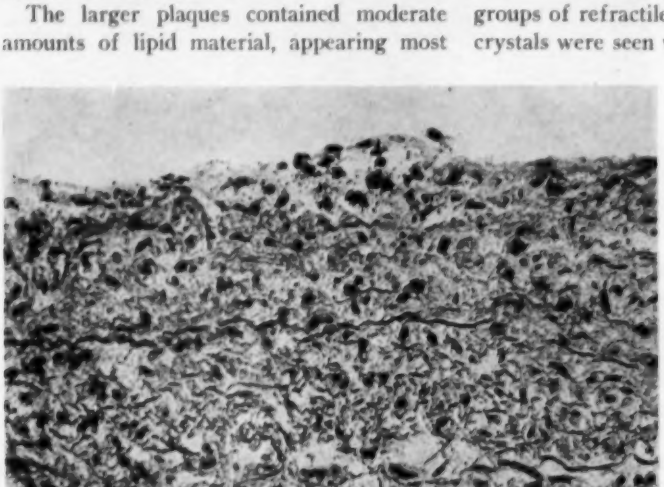

Fig. 9.—Thoracic aorta. Larger, coalescing lipid droplets fill the cytoplasm of the intimal connective tissue cells. Sudan IV-hematoxylin stain; reduced to 90% of mag. × 200.

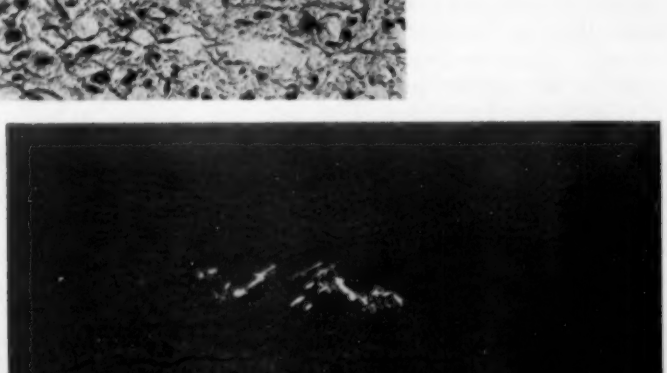

Fig. 10.—Thoracic aorta. Refractile crystalline deposit in midportion of intima. Polarized light; reduced to 90% of mag. × 100.

10). After gentle heating and pressure, these assumed a Maltese-cross pattern, characteristic of cholesterol or its esters.[9]

Abdominal Aorta.—Most of the circumference of the abdominal aorta displayed pronounced intimal thickening, especially on the posterior wall. Where the intimal widening was severest, distinct layering was noted (Fig. 11). The deeper intimal segments were compact and composed of irregularly arranged connective tissue cells and fibers. Usually the superficial portions appeared fibrillary and mucoid and contained fine, wavy fibers, lying near and parallel to the endothelial surface (Fig. 11). Here the cells were fewer, but were usually strikingly vacuolated. The internal elastic membrane beneath the thickened intima showed many points of fragmentation. Often the intimal connective tissue was extruded through these defects into the medial layer beneath. Bridging of some defects with reduplicated segments of internal elastic membrane was observed in many areas. The abnormal intima contained an abundant meshwork of mucopolysaccharide, reticulum, and collagenous fibers, mainly localized about the connective tissue cells and in the deeper, more compact segments of the thickened intimal layer. Many newly formed elastic fibrils appeared in layers, lying parallel to the endothelial surface. Elastic tissue appeared even in areas of minimal intimal thickening in the abdominal aorta. The elastic fibers were fibrillar, granular, and, where best developed, resembled the internal elastic membrane but were not hyaline or refractile. Usually, a layer of this substance lay beneath the endothelium, separated from the remainder of the intimal elastic tissue by the superficial mucoid portion of the intimal plaque (Fig. 11).

The intimal plaques contained moderate amounts of very finely divided lipid material, mostly applied to the elastic tissue fibers. This lipid was concentrated in the deeper portions of the plaques, where the elastic fibers were most numerous (Fig. 12). Larger, coalescing lipid droplets were present in the cytoplasm of the fibrocytes, although some larger droplets were extracellular. This lipid stained dark blue to dark violet with Nile blue but did not appear as refractile substance when viewed with polarized light.

Common Iliac Arteries.—In one animal intimal disease in these arteries was severer than in the aorta and consisted of intimal thickening involving the entire circumference of the vessels. Most of the intima con-

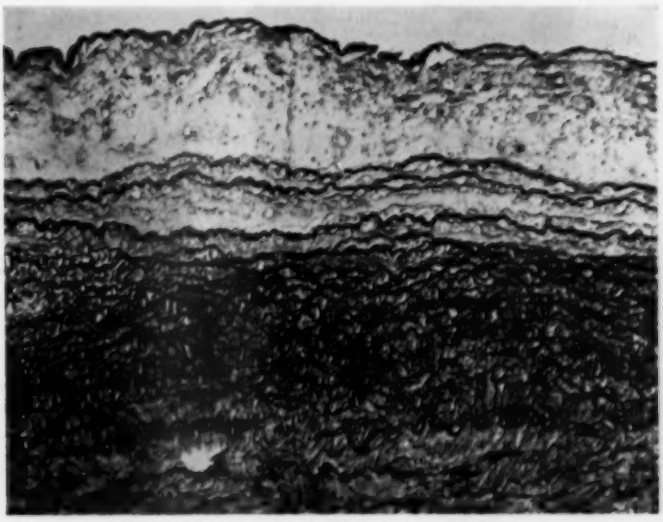

Fig. 11. — Abdominal aorta. The intima consists mainly of mucoid connective tissue. Note layers of elastic tissue in deeper portion of the intima. Weigert-Van Gieson stain; reduced to 90% of mag. × 200.

Fig. 12. — Abdominal aorta. The intimal lipid infiltration is localized to the elastic fibers. Sudan IV-hematoxylin stain; reduced to 90% of mag. × 200.

sisted of loosely arranged mucoid tissue, containing irregularly arranged fibrocytes and fibrils (Fig. 13). Where the thickening was greatest, the superficial portions tended to be more condensed and at times hyalinized, whereas the deeper segments of the thickened intima were generally mucoid or fibrillary. Some of the superficial intimal cells and adjacent endothelial cells had vesicular cytoplasm.

There was a rich network of fine mucopolysaccharide and reticulum fibers in the thickened intima, most numerous around the cells. Their arrangement was either perpendicular or parallel to the endothelial cells. The thickened intima also contained delicate collagenous fibrils, which were coarser and more condensed in the compact superficial segments of the thickened intima.

Extensive fragmentation, splitting, and reduplication of the internal elastic membrane beneath the thickened intima were observed. Newly formed elastic tissue was prominent in the thickened intima. In the

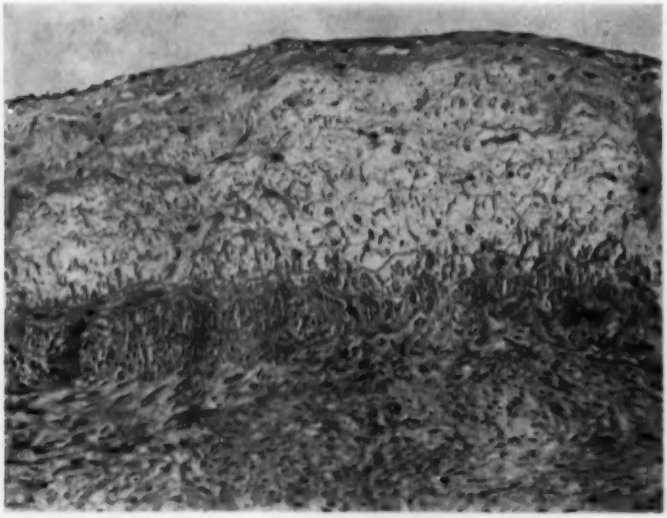

Fig. 13.—Common iliac artery. The intima consists of irregularly and loosely arranged fibroblasts and connective tissue fibers. Hematoxylin and eosin stain; reduced to 90% of mag. × 100.

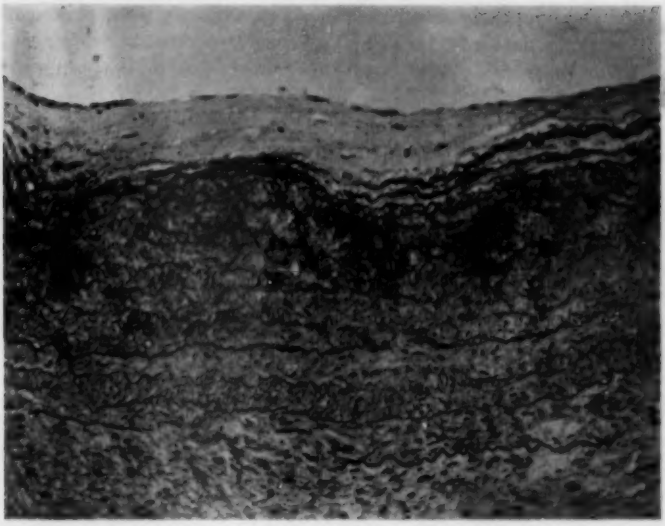

Fig. 14.—Common iliac artery. The superficial layer of the intima is devoid of lipid. The elastic fibers beneath are covered by small lipid droplets. Sudan IV-hematoxylin stain; reduced to 90% of mag. × 100.

deeper mucoid portions the fibers were delicate, fibrillary, and wavy, whereas in the compact superficial portions of the plaques the fibers appeared as coarse bundles of fibrillary elastic substance. Small amounts of amorphous mucopolysaccharide were concentrated about the elastic fibers.

Where the intimal thickening was minimal, no lipid material was found. Where the thickening was more pronounced, moderate amounts of lipid material were present. In the deeper layers, small lipid droplets were grouped in layers along the coarser elastic tissue fibers (Fig. 14). Smaller amounts of lipid were closely associated with the finer elastic fibers of the intimal plaques. In the superficial layers, larger, coalescing droplets were observed in the cytoplasm of the connective tissue cells. Some of the condensed superficial connective fibers stained slightly with Sudan IV, but distinct droplets were not present. All of the lipid was localized to the intimal layer, and none was present in the media. The fine droplets stained violet with Nile blue, whereas the larger, intracellular masses stained dark blue. In several areas in the midportion of the thickened intima, groups of elongated needle-shaped crystals, arranged radially, were visible with polarized light. These assumed a Maltese-cross pattern, following heating and pressure.[9]

Comment

Arteriosclerosis in the baboon is initiated by degeneration of either the internal elastic membrane or inner layer of the medial elastic tissue and by deposition of increased amounts of mucopolysaccharide substance. Regeneration of the damaged elastic tissue, by reduplication of the elastic layers and development of new layers derived from the ground substance, is identical with the arteriosclerotic process observed in other species.[10-13] Proliferation of fibroblasts and formation of mucopolysaccharide, reticulum, and collagenous and elastic fibers by the connective tissue cells lead to the development of intimal thickening and formation of intimal plaques. The sequence of events and the appearance and arrangement of the constituents of the altered intima are the same as those observed previously in the arteries of birds, dogs, cats, and human beings.[10-15]

Although lipid substance was demonstrable in some of the earlier lesions, its deposition seemed to be preceded by degenerative disease of the elastic tissue. Deposits of lipid occurred mainly near

degenerating elastic tissue, and the lipid had an apparent affinity for the accumulating mucoid ground substance. After the intima had thickened, or after intimal plaques had developed, the lipid was found closely applied to the newly developed intimal elastic fibers, and here seemed to have a greater attraction for elastic tissue than for mucoid ground substance. Some of the lipid droplets accumulating in this fashion coalesced and appeared within the cytoplasm of the intimal fibrocytes. Phagocytic mononuclear cells or foam cells were not found in these lesions. Although the bulk of the lipid was probably neutral fat, small amounts of cholesterol substances were demonstrated. The nature of the lipid deposited in the thickened intima and its appearance following the degeneration and proliferation in the intima are the same as those observed previously in the bird, dog, and cat.[10-13] Cholesterol, however, was not observed in the lesions of the dog and cat.[10-12]

It is of special interest that our two baboons had been fed a diet low in fats and probably devoid of cholesterol. The levels of plasma cholesterol were not determined in these two baboons, but in a group of eight young male baboons (Papio anubis) the concentrations of plasma cholesterol ranged from 90 to 140 mg. per 100 cc.[16] Van Zyl and Kerrich [17] and van Zyl [18] also measured the levels of serum cholesterol in baboons (Papio ursinus) and reported values of 118 to 250 mg. per 100 cc. Our finding that the intimal lipid was concentrated mainly along regenerating elastic fibers generally in the deeper portions of the plaques suggests that this intimal lipid may not have been transported from the lumen by diffusion but, rather, was synthesized in situ. That the normal arterial wall of various animals is capable of synthesizing fatty acids and cholesterol has been demonstrated.[19-22]

The naturally occurring arteriosclerosis described here in the baboon is fundamentally similar to the lesions found in other primates by Fox,[1] Steiner,[2] and Rinehart and Greenberg.[6,7] The lesions described by these other investigators were primarily fibrous and mucoid, showed elastic degeneration, and contained no or insignificant amounts of lipid material. However, the lesion found in the cebus monkey by Mann and his co-workers [5] represented a different sort of process; in that case the intimal thickening resulted from accumulations of macrophages or foam cells filled with lipid substance, secondary to hypercholesteremia. This is the same type of lesion which has been observed repeatedly in the cholesterol-fed rabbit,[23] in the cholesterol-fed bird,[13] and in the hypercholesteremic dog.[11,24,25] This lesion is primarily lipid in origin and has little or no resemblance to that occurring naturally in the human being. Mann and his co-workers [8] presented no histologic evidence to indicate that a diet deficient in protein had results in degenerative vascular disease similar to the naturally occurring process that is not associated with hypercholesteremia.

Summary

Arteriosclerosis of the aorta and coronary and common iliac arteries of the Doguera baboon (Papio anubis) is described.

The arterial lesion is characterized by an initial degeneration of the internal elastic membrane or inner medial elastic layer, followed by localized deposition of mucopolysaccharide substance.

Intimal fibrosis follows, with formation of elastic, reticulum, and collagenous fibers, leading to the development of concentric intimal thickening or intimal plaques.

Lipid infiltration of the intimal lesions is minimal and appears secondarily to intimal degeneration and fibrosis. Foam cells are not observed in the arteriosclerotic lesions, but small cholesterol deposits are demonstrable deep in the intimal plaques.

Drs. C. R. Schroeder and A. W. Sylstra supplied the baboon tissues. Mrs. Thelma Gotham and Mr. Hal Strong aided in the preparation of the sections and photographs.

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Experimental Arthritis

I. Morphologic Alterations in the Guinea Pig After the Parenteral Injection of Bacterial Extracts

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During studies on rheumatic-like lesions in the guinea pig, a wide variety of bacterial and animal products as well as chemical substances were tested and certain substances classifiable as mucopolysaccharides were found to induce synovial proliferation with or without accompanying joint exudate.[1,2] A spontaneous, as well as induced, infection with a Klebsiella organism was also found to be accompanied by joint lesions, and a crude polysaccharide extracted from this organism and injected subcutaneously or intravenously into guinea pigs also induced joint lesions. In a further study of the pathogenesis of the joint lesions, a known Type B Klebsiella pneumoniae organism was used and the effects of several different extracts from this organism were compared.[3] The arthropathic effect was not attributable to lethal toxicity, anaphylactogenic properties, or differences in protein, nitrogen, hexosamine, hexuronic acid, or mono- or disaccharide content.[3] In the latter studies, the histologic changes in the joints were evaluated after repeated daily subcutaneous and intravenous injections. A number of animals dying during the first 24 hours after the injection of the lethal extract were found to have joint lesions. Experiments were therefore undertaken to study the early phases of development of the joint lesions and to inquire further into the specificity of this response.

The present paper concerns *(a)* the histogenesis of the joint lesions during the first few hours and days after a single intravenous dose; *(b)* the arthropathic effect of certain other bacterial extracts, and *(c)* the morphologic changes in the viscera of guinea pigs given the various bacterial products.

Methods and Materials

Bacterial Products.—The somatic antigen of Shigella paradysenteriae Type Z and five extracts and two "residues" of K. pneumoniae Type B were studied. K. pneumoniae Type B organisms incubated 18 hours at 37 C on agar plates containing 1% dextrose and 4% peptone[4] were scraped from the agar, suspended in distilled water, and extracted by three different procedures—the alkaline- and acid-extraction techniques of Wong[5] and the trichloroacetic acid method of Jancsik and Kaiser[6] for hyaluronic acid in tissue. The last procedure employs 4% trichloroacetic acid adjusted to pH 4.0 with sodium acetate, three-day refrigeration, and ethanol precipitation. In addition, bacterial debris remaining after the alkaline and acid extraction were injected into animals.

K. pneumoniae organisms were also grown on deferrated agar medium prepared by the method of van Heyningen[7,8] and the crude polysaccharide extracted by the acid procedure of Wong.

A purified capsular polysaccharide of Type B K. pneumoniae and the somatic antigen of Type Z S. paradysenteriae obtained through the courtesy of Dr. Walther Goebel[9,10] were also utilized in these experiments.

All of these bacterial products were dissolved as a 1% solution in 0.85% sodium chloride and sterilized by autoclaving. Only the water-soluble fractions of the bacterial residues were injected.

Animals.—Young guinea pigs of either sex and weighing 175-250 gm. were given water and Purina rabbit chow ad libitum and daily supplements of

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TABLE 1.—*Joint Changes and Bile Precipitate Observed After Several Daily Intravenous Injections of Bacterial Extracts* *

	Daily Intravenous Dose	Two Injections Killed Day 4			Four Injections Killed Day 6		
			Joint			Joint	
		Bile Precipitate	Synovial Prolif.	Exudate	Bile Precipitate	Synovial Prolif.	Exudate
Control, 0.85% saline	1 ml.	0/4	0/4	0/4	0/4	0/4	0/4
Somatic antigen S. paradysenteriae Type Z	0.5 mg.	1/4	4/4	4/4	3/4	4/4	3/4
Purified capsular polysaccharide K. pneumoniae, Type B	5.0 mg.	0/2	0/2	0/2	0/4	0/4	0/4
TCA-extracted polysaccharide K. pneumoniae, Type B	2.0 mg.				3/4	4/4	4/4
Acid-extracted polysaccharide K. pneumoniae, Type B from deferrated medium	1.0 mg.				4/4	4/4	4/4

* Numerator indicates number of animals with change; denominator indicates number in the particular group.

cabbage throughout the experiments. For the study of the histogenesis of joint lesions, the acid- and alkaline-extraction polysaccharides of K. pneumoniae were given as a single 5 mg. injection into the transilluminated ear vein. Three guinea pigs were killed at each of the following intervals: 30 minutes; 1, 3, 6, and 12 hours, and 1, 3, 7, and 14 days. For comparison with the intravenous route, 5 mg. of the alkaline-extraction polysaccharide was injected intraperitoneally and animals killed at the above time intervals.

For the study of arthropathic and toxic qualities of these products and of the bacterial debris, trichloroacetic-acid-extracted polysaccharide, and acid-extracted polysaccharide from deferrated media, animals were killed two days after four daily injections of 2, 2, and 1 mg. per day, respectively. Five milligrams of purified K. pneumoniae capsular polysaccharide and 0.5 mg. of the somatic antigen of S. paradysenteriae were given intravenously to separate groups for two and four consecutive days and the animals killed two days after the last injection.

After killing the guinea pigs, the extremities were fixed in acetone-formaldehyde and microsections of the femorotibial (knee), scapulohumeral (shoulder), and humeroulnoradial (elbow) joints were stained with hematoxylin and eosin, toluidine blue, and, in order to evaluate the nature of the exudate, with the periodic acid-leukofuchsin (PAS), phosphotungstic-acid hematoxylin, and Mallory aniline blue stains. Routine sections of the brain, salivary glands, cervical and mesenteric lymph nodes, trachea, lungs, heart, spleen, liver, gall bladder, pancreas, adrenals, kidneys, and gonads were examined.

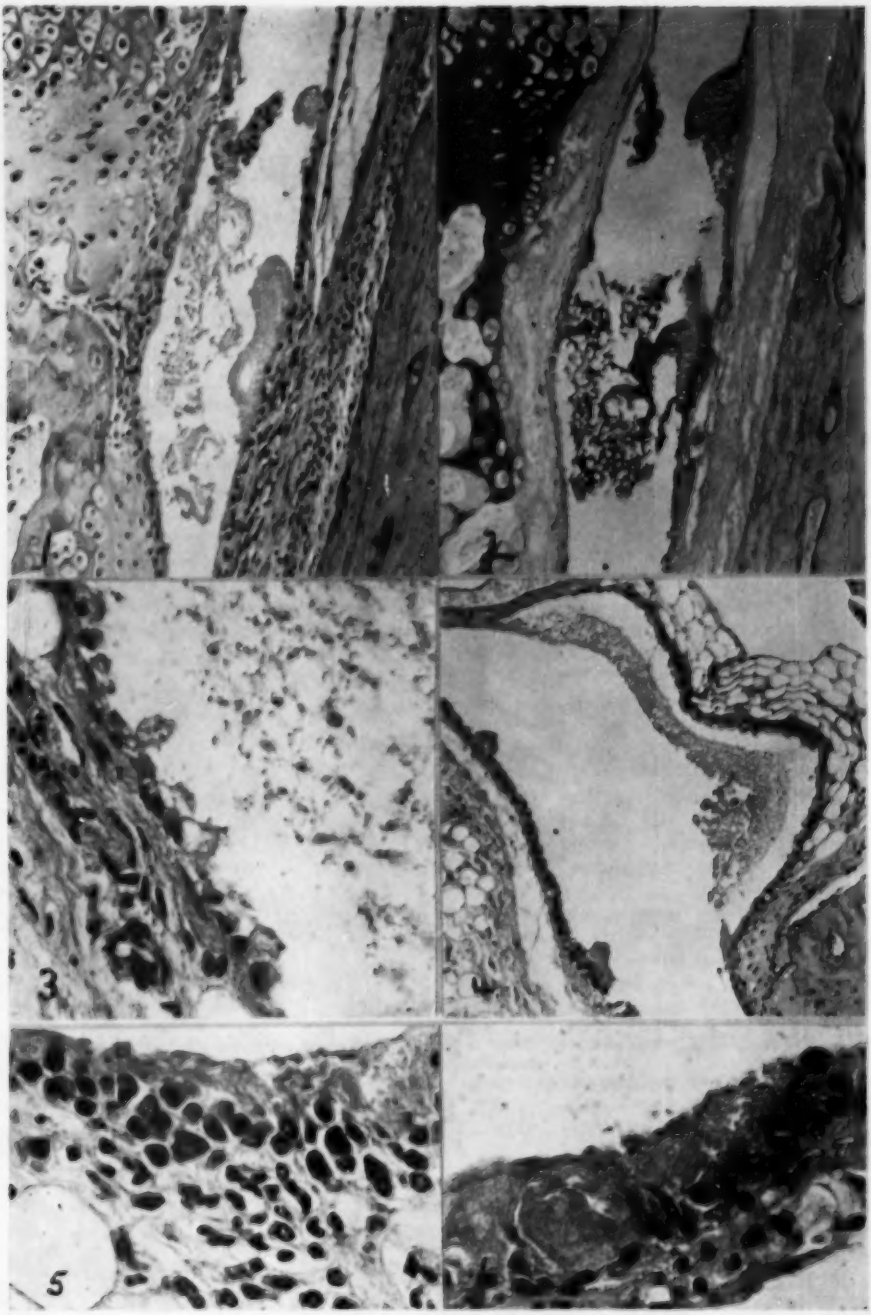
Results

Induction of Joint Lesions.—All of the bacterial substances in this experiment, except the purified K. pneumoniae capsular polysaccharide, produced joint changes (Tables 1 and 2). The lesions induced with the daily doses of the highly toxic somatic antigen of Type Z S. paradysenteriae were as severe as those produced by a single injection of a larger quantity of toxic, acid-

TABLE 2.—*Tissue Changes Following a Single Intravenous Injection of 5 Mg. of K. Pneumoniae Extracts* *

		½ Hr.	1 Hr.	3 Hr.	6 Hr.	12 Hr.	1 Day	3 Days	7 Days	14 Days
Acid-extracted polysaccharide K. pneumoniae	Ascites	0/3	0/3	2/3	3/3	3/3	1/3	0/3	0/3	0/3
	Retroperitoneal fluid	0/3	0/3	1/3	3/3	3/3	1/3	0/3	0/3	0/3
	Bile precipitate	0/3	0/3	0/3	3/3	3/3	3/3	2/3	2/3	1/3
	Hepatic basophilia	0/3	2/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3
	Joint mucoid increase	0/3	1/3	3/3	2/3	0/3	1/3	1/3	0/3	0/3
	Small globules	0/3	0/3	2/3	1/3	2/3	1/3	0/3	1/3	0/3
	Synovial proliferation	0/3	0/3	0/3	0/3	1/3	3/3	3/3	3/3	0/3
	Exudate	0/3	0/3	0/3	0/3	3/3	3/3	3/3	2/3	0/3
Alkaline-extracted polysaccharide K. pneumoniae	Ascites	0/3	0/3	0/3	1/3	0/3	2/5	0/5	0/5	0/3
	Retroperitoneal fluid	0/3	0/3	0/3	Slight 0/3	1/3	Slight 2/5	0/5	0/5	0/3
					Slight	Slight	Slight			
	Bile precipitate	0/3	0/3	0/3	0/3	0/3	0/5	0/5	0/5	0/3
	Hepatic basophilia	0/3	0/3	0/3	0/3	0/3	0/5	0/5	0/5	0/3
	Joint mucoid increase	0/3	2/3	2/3	2/3	1/3	2/5	0/5	0/5	0/3
	Small globules	0/3	1/3	1/3	3/3	0/3	1/5	0/5	0/5	0/3
	Synovial proliferation	0/3	0/3	0/3	1/3	1/3	3/5	4/5	4/5	0/3
	Exudate	0/3	0/3	0/3	Slight 2/3	1/3	3/5	3/5	4/5	0/3

* Numerator indicates number of animals with change; denominator indicates number in the particular group. Animals dying during the first day after the injection of the acid-extraction polysaccharide are not included. A joint change is listed as positive if observed in only one of the five or six joints examined per animal.



Figs. 1-3.—Sections are from elbow joint of guinea pig injected intraperitoneally five hours previously with 5 mg. of alkaline-extraction polysaccharide. Earliest joint changes are an apparent increase in mucoid joint fluid (Fig. 1; hematoxylin and eosin; × 100), which stains metachromatically (Fig. 2; toluidine blue; × 100). Under higher magnification (Fig. 3) the synovial cells appear vacuolated and swollen but contain only a few minute metachromatic granules; synovial fluid shows ovoid or spheroid particles with mild metachromatic staining (× 300).

Figs. 4-6.—Elbow joint of guinea pig killed 24 hours after intravenous administration of 5 mg. of alkaline-extracted polysaccharide. Synovial cells have proliferated, and the joint fluid contains discrete orthochromatic, eosinophilic masses (Fig. 4; × 100). Less homogeneous, but similar, material covers and merges with the cytoplasm of the proliferated synovial cells (Fig. 5; × 300). This process is more prominent in Figure 6 (× 300).

extracted polysaccharide prepared from organisms grown on iron-free media. The alkaline-extraction polysaccharide produced somewhat less severe lesions, although it had no lethal or other toxicity.

In general, the elbow joints show the most change and the shoulders the least. This parallels the relative amount of cuboidal or columnar synovial cells in elbow, knee, and shoulder joints of the control, or saline-injected, animals.

Histogenesis of Joint Lesions.—The histologic changes within the joints consist of increase in synovial cells and the appearance of an eosinophilic exudate within the joint space and upon the synovial epithelium. The histogenesis is reconstructed by the comparison of changes in animals killed at the various intervals after a single intravenous injection.

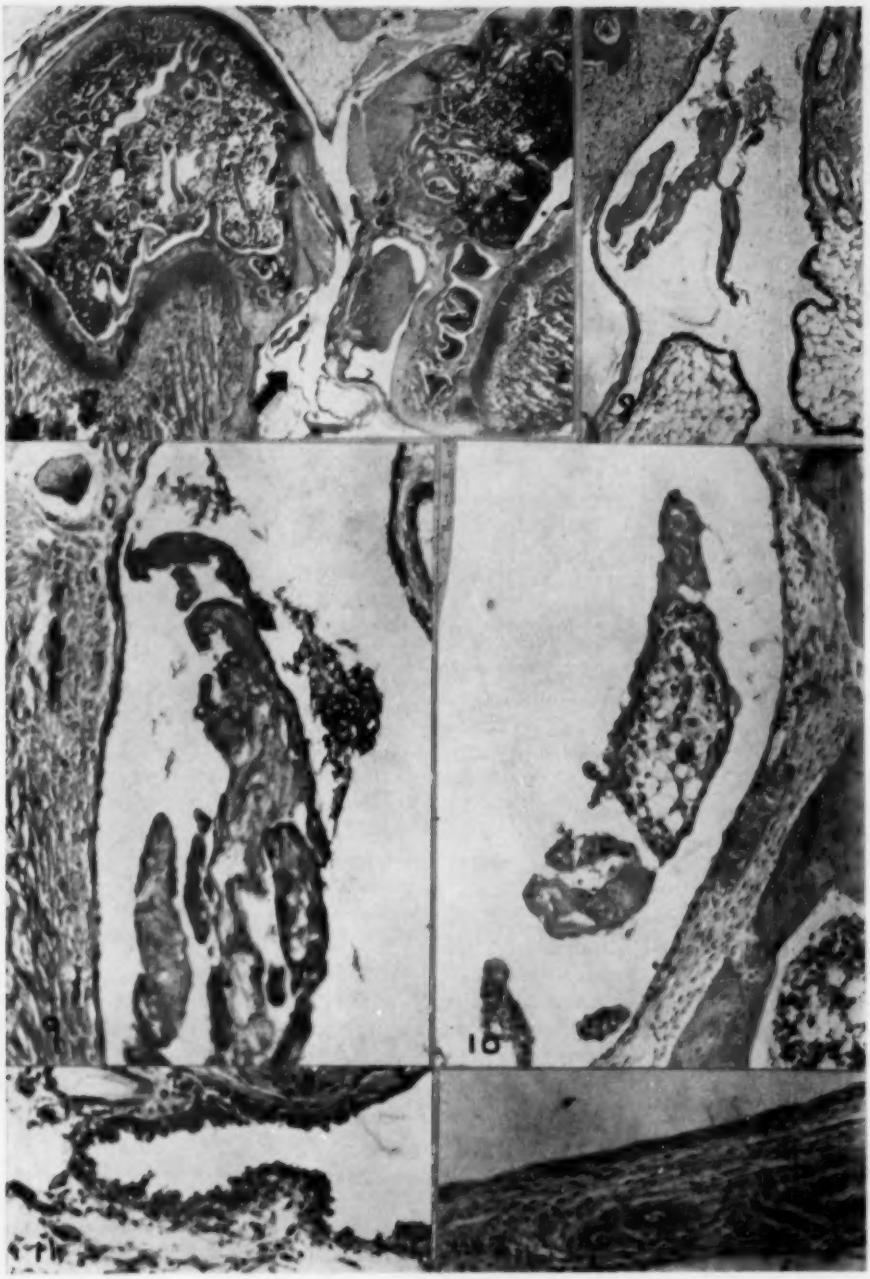
Within one hour after the intravenous injection of the injurious agent, there is an increase in the mucoid material in the joint cavity. Although not quantitatively evaluated, the mucoid material is most abundant within the elbow joints. After the acetone-formalin fixation, this material has a finely granular and fibrillar appearance (Fig. 1). Within three to six hours, small, oval to round globules, easily distinguished from a rare erythrocyte, are noted within the mucoid substance (Fig. 3). In contrast to the mucoid material, these small globules have but mild metachromatic staining. About the same time, some of the synovial cells are vacuolated and have irregular or frayed free borders (Fig. 3), but do not contain metachromatic material. This occurs especially within the elbow joints opposite the articulating cartilage and remains as long as three days. By 6 to 12 hours the globules within the joint space are much larger, occasionally appearing intimately associated with the synovial cells (Fig. 5). After a few days, however, synovial cells have become larger and more numerous and have a dense, nonvacuolated eosinophilic cytoplasm. Often, the synovial cells have an associated overlying layer of eosinophilic, slightly granular material. The synovial cytoplasm merges indistinguishably into the eosinophilic material. Rarely a small mass of eosinophilic exudate lies beneath the epithelium. Regardless of location, this exudate does not stain as fibrin with phosphotungstic-acid hematoxylin or Mallory's aniline blue, suggesting possible origin from the synovial cytoplasm. This process continues for one week, then disappears without recognized residue.

Occasionally during the first few hours after an intravenous injection one may see a few polymorphonuclear leukocytes in the subsynovial area, and occasionally a few of these cells, as well as monocytes, may be within the mucoid substance of the joint space. During the active synovial proliferation there is only a mild increase in fibroblasts and macrophages in the adjoining connective tissue. Changes in surface layers of the articulating cartilage may occur but are difficult to distinguish from artifactual separation of thin surface strips and fragments.

Changes in Other Tissues.—All of the bacterial products except the alkaline-extracted Klebsiella polysaccharide and Goebel's purified capsular polysaccharide possess lethal properties. These same substances also produce changes in the bowel, liver, and gall bladder of the surviving animals.

After the injection of the lethal toxic materials, the animals often appear ill during the first 12 hours, assuming a hunched position with ruffled fur and refusing food and water. Death usually does not occur for 12 to 24 hours.

Protein-rich ascitic fluid is present in small quantity at 6 hours, is most pronounced at 12 hours, and is usually absent at 24 hours in killed animals. As a concomitant process, a jelly-like, coagulated exudate is noted in the retroperitoneal tissues, especially about the pancreas (Fig. 18) and renal pelves. Microscopic evidence of retroperitoneal edema may appear as early as three hours. Occasionally, some fluid ac-



Figs. 7-12.—Exudate is present in synovial space of knee joint (arrow) of animal given bacterial mucopolysaccharide (Fig. 7; × 10), and with higher power (Fig. 8; hematoxylin-eosin stain; × 40) accompanying increase in synovial cells is seen. Toluidine blue stain (Fig. 9; × 110) reveals metachromatic synovial mucoid clinging to surface of exudate. In Figure 10 the exudate is intimately associated with the surface of the proliferated synovial cells (× 110). The increased synovial cells are not accompanied by significant changes in adjacent connective tissue (Fig. 11; × 110), although in some areas (Fig. 12; × 110), fibroblasts and macrophages may be mildly increased. Guinea pig was given two daily intravenous injections of 0.5 mg. of S. paradysenteriae Type Z somatic antigen and killed two days after the last injection.

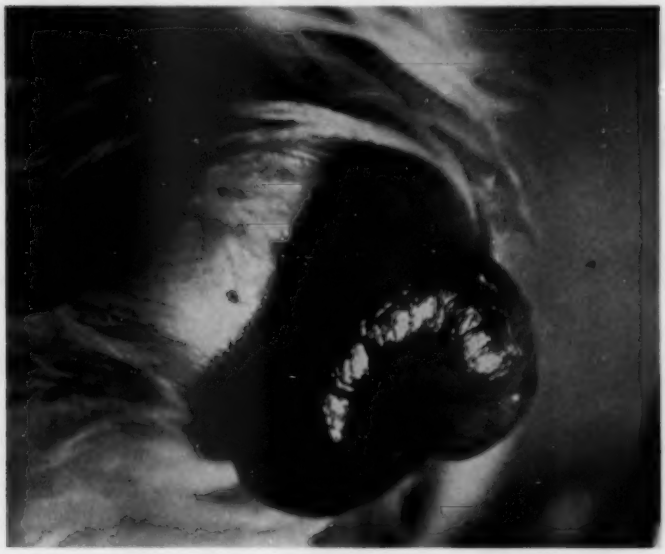


Fig. 13.—In a few guinea pigs injected with toxic bacterial products the intussuscepted rectosigmoid bowel may prolapse through the anus.

cumulates in the pleural and pericardial cavities. With the acid-extracted polysaccharide from organisms grown on deferrated media, the peritoneal fluid is bloody. The surviving animals regain their normal activity and appetite, paralleling the disappearance of peritoneal and retroperitoneal exudate.

With the intravenously injected nonlethal, alkaline-extracted polysaccharide from K. pneumoniae, there are few manifestations of illness, but a few animals killed at 12 hours disclose a trace of peritoneal and retroperitoneal exudate. Intraperitoneal injection of the same 5 mg. quantity as given intravenously results in pronounced fluid accumulation of the peritoneal cavity with occasional death of the animals.

During the first day after the intravenous injection of the toxic material, a viscid mucus appears at the anus in a few guinea pigs, and by the second day the intussuscepted rectosigmoid bowel prolapses through the anus (Fig. 13). Death of these animals may sometimes be averted by the early reduction of the intussusception.

Bile precipitate in the gall bladder and extrahepatic bile ducts (Fig. 14) also results from the injection of lethal toxic materials or from the intraperitoneal, but not intravenous, injection of the alkaline-extracted polysaccharide of K. pneumoniae. The flocculent, greenish bile precipitate appears as early as six hours and is associated with obvious distention of the gall bladder. Microscopically, the bile appears to cling to detached mucosal epithelia. The bile precipitate may be present as long as two weeks after a single intravenous injection of toxic material, gradually becoming lighter in color. In some animals receiving repeated injections a small firm, whitish calculus may form.

Within one to three hours after the intravenously injected toxic extracts and the nonlethal alkaline extract introduced intraperitoneally, the hepatic cord cells contain rounded, nonmetachromatic particles, which stain bright blue with hematoxylin and eosin (Fig. 15) and red with PAS and disappear with diastase digestion. These particles increase in size and number, and by 24 hours large, irregular masses may occupy much of the cytoplasm. The basophilic material may persist one week, then disappear. No changes are seen in the Kupffer cells, and there is no leukocytic infiltration.

Fig. 14.—Flocculent bile precipitate occurring in the gall bladder has been expressed without apparent resistance into the extrahepatic bile ducts.

The variability in histologic appearance of lymph nodes makes evaluation difficult, but within a few hours there is a less compact arrangement of follicular germinal centers and sinusoids are wide and occasionally contain a pale, eosinophilic material, a few erythrocytes, and phagocytes with engulfed erythrocytes. This alteration is more pronounced in the cervical lymph node after the intravenous injection of acid- or alkaline-extracted polysaccharide and more marked in mesenteric lymph nodes after intraperitoneal injection. Within 24 hours the sinusoidal phagocytes become more prominent (Fig. 17), and cells resembling plasmacytes are more numerous.

The adrenal cortex, the site of an unusually high concentration of C^{14} from injected labeled polysaccharide,[11] shows but minor change with the present histologic techniques[3]; during the first day it is often grossly reddened and microscopically shows engorgement, a few small hemorrhages, and dissolution of a few cortical cells.

Comment

The joint lesions, bile precipitate, ascites, and basophilia of hepatic parenchymal cytoplasm following the injection of extracts of K. pneumoniae and S. paradysenteriae might also be expected with many Gram-negative bacilli. Such substances have long been employed in the production of the Shwartzman phenomenon, but only hemorrhages, thromboses, and necroses in various organs have been described. In studying the Shwartzman phenomenon, Apitz[12] observed focal necrosis in the liver, spleen, and heart of rabbits, but not of guinea pigs, after the two intravenous injections of filtrates of enteric organisms or Meningococcus. Toxic substances extracted by Boivin's[13] technique from Salmonella typhi and given as a single injection produced necroses in the liver of rabbits,[14] similar to those observed in typhoid in man.[15] Martin[16] after injecting mice intraperitoneally with Raistrick's[17] Salmonella aertrycke (S. typhimurium) extracts, observed engorgement of peritoneal vessels and of the adrenal; in the adrenal there was a striking increase in mononuclear, and sometimes polymorphonuclear, leukocytes, along with degeneration of the medullary cells. Gerber[18] found a few thromboses in the lung of rabbits after a single injection of S. typhi filtrate while thromboses in various organs followed a single injection of Neisseria meningitidis fil-

trate. Morgan [19] observed similar thromboses and necroses in rabbits after a single intravenous injection of toxic somatic antigen from S. typhi.

Studies on the morphologic changes have been meager as compared with the investigations of chemical, toxic, and antigenic properties of the Gram-negative organisms.

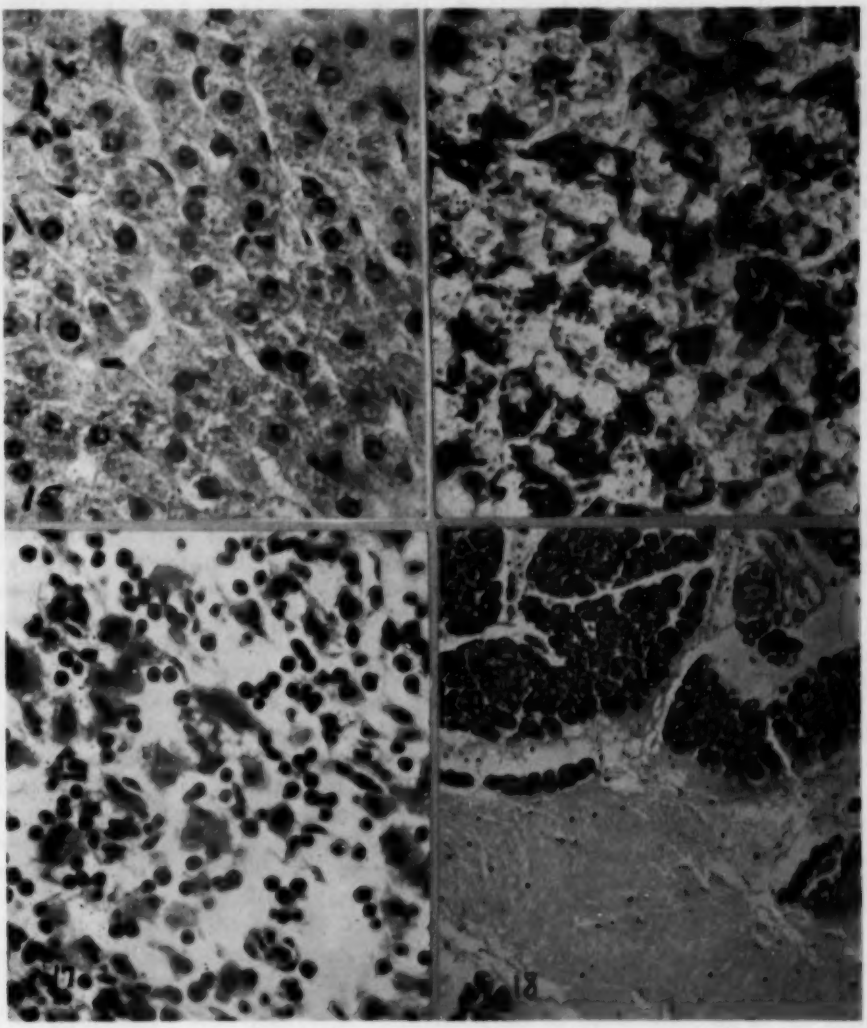


Fig. 15.—One hour after the intraperitoneal injection of 2 mg. of alkaline-extraction polysaccharide of K. pneumoniae, the hepatic cord cells contain small rounded basophilic particles. Hematoxylin and eosin; × 300.

Fig. 16.—Within a few hours, large basophilic masses appear in the cord cells and stain intensely with periodic acid-leukofuchsin (PAS). This alteration occurs after intravenous injection of lethally "toxic" bacterial products or after the intraperitoneal injection of nonlethal alkaline-extraction polysaccharide (PAS; × 300).

Fig. 17.—Reticuloendothelial cells of the lymph node are prominent in number and size, especially in the cervical nodes after intravenous injection and in mesenteric nodes after intraperitoneal injection of bacterial products. Hematoxylin and eosin; × 300.

Fig. 18.—There is protein-rich fluid about the pancreas in guinea pig killed 14 hours after intraperitoneal injection of 5 mg. of alkaline-extraction polysaccharide. Hematoxylin and eosin; × 100.

Various investigators, employing many different techniques, have obtained complexes of polysaccharide-lipid-protein which are heat-stable, toxic, and antigenic and which characterize the surface of the bacteria in their virulent, or smooth, form. These complexes have been dissociated into various components in an attempt to identify the antigenic, haptenic, and toxic fractions. Boivin [18,20] extracted an immunizing toxic polysaccharide from S. aertrycke which could be separated with acetic acid into a nontoxic, nonantigenic polysaccharide hapten and a toxic, nonhaptenic substance thought to be a phosphatide. Raistrick and Topley[17] obtained similar toxic, immunizing polysaccharide complexes whose nitrogen-containing component was thought to be a peptide rather than a protein.

Extensive studies of this type carried out by Morgan and Partridge [21] on S. dysenteriae (Shiga) and Goebel [19] on S. paradysenteriae Type Z indicate that the phospholipin is unnecessary for toxicity or serologic activity, but the chemical component or physicochemical aspects responsible for toxic manifestations have not been clearly established. "Purity" of the various fractions has been difficult to obtain when all criteria are used. Cluff [22] isolated S. paradysenteriae Type Z antigen by Goebel's technique [19] and found three electrophoretic fractions with a common, as well as distinct, components on gel-precipitin techniques.

In understanding the pathogenesis of the joint lesions and other tissue changes, several questions may be posed: *(a)* What is the role of "toxicity"? *(b)* Does a single physiologic or metabolic alteration explain all of the host responses? *(c)* Are the changes due to the direct effect of the injected material, or are they produced indirectly through other metabolic or physiologic alterations of other organs or tissues?

The "toxicity" of materials injected into animals implies only the ability to produce death or such general manifestations of illness as listlessness, anorexia, fever, hemorrhage, diarrhea, and fluctuations in respiratory activity, cardiac rate, blood sugar, and blood leukocyte count. Delineation of "toxicity" in more exact physiologic and metabolic terms is needed. However, within the present limitations, it does appear that a nonspecific and nonimmunologic tolerance to the toxic substances may be acquired. Febrile responses are markedly diminished with repeated injections in the rabbit and are unassociated with a specific antibody, since the tolerance *(a)* disappears when agglutinins are present in serum, *(b)* follows the injections of nonantigenic bacterial pyrogens, and *(c)* cannot be passively transferred.[23] In addition, antiserum does not neutralize the toxic effects in animals,[24,25] and the precipitate from antigen-antiserum reactions still possesses toxicity.[26] A previous study correlating the morphologic changes with toxicity suggested a lethal toxicity distinct from arthropathic properties.[3] The present investigation does not support this; the nontoxic arthropathic alkaline extract, when given intravenously, is toxic upon intraperitoneal injection. Further experiments are needed to elucidate the effects upon "toxic response" resulting from variations in distribution of injected material, in absorption to plasma proteins or cell surfaces, and in alterations of physicochemical state.

Dennis [14] believed the effects of typhoid toxin in animals might be due to vascular injury. The neurotoxin of S. dysenteriae is thought to produce destructive lesions in the central nervous system not by direct action on nerve cells but by injury of the endothelium of smaller blood vessels.[27,28] The increased capillary permeability from the various toxic extracts used in the present study would permit the exudation of both plasma protein and the toxic colloidal material and led to the ascites, bile precipitate, joint lesions, and even death. Different rates of removal of the foreign material from serous and synovial spaces may account for the relatively rapid disappearance of protein-rich ascitic fluid, while the joint lesions persist and progress.

Death from the toxins of Gram-negative bacilli does not occur for 6 to 24 hours after

injection. This may be due to the direct, although gradual, effect of the injected material upon capillary walls, or, on the other hand, to changes brought about indirectly by antecedent injury to other organs or tissues. The indirect action of the injected material is given some credence by unpublished observations that the injection of 1 ml. of serum removed from toxic animals induces all of the lesions more rapidly and in comparable severity. By C^{14} labeling of the bacterial extract, it is known that the bacterial product in the reinjected serum is negligible.[29]

A possible relationship between the hepatic cytoplasmic basophilia and the hyperglycemia produced by Gram-negative organisms is of interest. Delafield [30] observed that the intravenous injection into rabbits of dead cells of many Gram-negative, but not Gram-positive, organisms leads to toxic effects and hyperglycemia. Delafield [31] also observed that antigenic, somatic polysaccharides from S. aertrycke [17] produced hyperglycemia; that all of the toxic manifestations are not attributable to altered carbohydrate metabolism is suggested by an acid-hydrolyzed material which was toxic but did not produce hyperglycemia. The present observation of basophilic particles and masses in the hepatic cytoplasm may be a morphologic stigma of altered carbohydrate metabolism. These masses are positive with PAS and disappear with diastase treatment—presumptive evidence of polysaccharides, such as glycogen. Whether altered carbohydrate metabolism in liver, muscle, and other tissues has any role in the pathogenesis of the morphologic changes, especially the joint lesions, is unknown.

While the toxic substances from Gram-negative organisms produce both the joint lesions in the guinea pig and the Sanarelli, or "generalized Shwartzman," phenomenon in rabbits, there is no morphologic evidence of a similar or common pathogenesis. Occlusive vascular lesions, predominantly in small arteries and capillaries,[12,18,32] with accompanying ischemic necroses in the Shwartzman reaction of rabbits, did not occur in the guinea pig after two spaced intravenous injections [12] or in the present study with a single or repeated injections. Histologically, there is no apparent similarity between the local Shwartzman reaction with necroses, thromboses, hemorrhages, and leukocytic exudation about the synovia [33] and the lesions following a single intravenous or intraperitoneal injection in the guinea pig. Brunson and co-workers [34] suggest that the Shwartzman response may result from changes in the circulating blood, perhaps from removal of fibrinogen with localization of blood-derived fibrinoid at sites of injury. Similar changes in the agent or circulating blood, as yet undefined, may form a common basis for the lesions in the guinea pig and the Shwartzman response in rabbits.

Summary

Acute joint lesions have been induced by the injection of antigenic polysaccharide complexes from Shigella paradysenteriae Type Z and Klebsiella pneumoniae Type B. Within a few hours after a single injection of toxic materials there is an apparent increase in metachromatic mucoid material in the joint cavity; small orthochromatic globules appear, synovial cells become frayed and vacuolated, and within 24 hours the synovial cells have increased in number and size. An eosinophilic exudate also appears but does not stain as fibrin. Within one or two weeks after a single intravenous injection these lesions have disappeared. Changes in other tissues consist of a protein-rich, noncellular ascitic and retroperitoneal fluid, a flocculent precipitate of bile in the gall bladder, and coarse basophilic granules in the hepatic cord cells.

The pathogenesis of the joint lesions is discussed in relation to "toxicity," chemical properties of the injected compounds, capillary permeability, and the Sanarelli, or generalized Shwartzman, response in rabbits.

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Experimental Arthritis

II. Studies with C^{14}-Labeled Polysaccharide Complexes of Klebsiella Pneumoniae, Type B

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In prior experiments "rheumatic-like" cardiac valvular and joint lesions have been produced by a wide variety of bacterial and chemical agents.[1] Crude polysaccharides and other fractions of Type B Klebsiella pneumoniae,[2,3] as well as the somatic antigen of Shigella paradysenteriae Type Z,[3] produced acute arthritic lesions after intravenous injection. In the study of the pathogenesis of rheumatic-like lesions, it is important to determine the distribution and metabolism of exogenous inciting agent or agents. Various fractions of K. pneumoniae Type B, labeled with C^{14} by biosynthesis and injected intravenously into guinea pigs,[4] have a distribution and duration comparable to that obtained by other techniques, such as those with colloidal materials[5,6] or with antigens tagged with dyes[7] and I^{131} [8-10] or by fluorescent dye-antibody complexes.[11-13] However, with the C^{14}-labeled crude polysaccharide of K. pneumoniae, the adrenal had the greatest incorporation of C^{14} per unit weight of any tissue, and C^{14} remains in fair concentration in the adrenal for two months, while it disappears from other organs.[4]

The present study of the acute arthritic lesions in the guinea pigs concerns the tissue distribution, metabolism, and nature of incorporation of two arthropathic polysaccharide complexes of K. pneumoniae, employing isotopic tracer, autoradiographic, extraction, and immunologic procedures.

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Methods and Materials

Bacterial Polysaccharides.—K. pneumoniae Type B organisms were grown on peptone agar[2] containing 7.7μc to 67.0μc of 1-C^{14} acetate per milliliter of medium. After alkaline- and acid-extraction techniques of Wong,[14] the crude polysaccharides have activities of from 0.16μc to 1.3μc per milligram. The acid- and alkaline-extracted polysaccharides have, respectively, a 39.8% and 34.6% reducing sugar content after hydrolysis with 1 N HCl for 16 hours at 100 C, a 3.6% and 4.3% nitrogen content, and a 7.5% and 6.8% protein content. For injection into animals, the polysaccharides are dissolved as a 1% solution in 0.85% NaCl and sterilized by autoclaving. Under these conditions, the intravenously injected alkaline-extracted polysaccharide is not lethal up to 80 mg. per kilogram of body weight, while the L. $D._{50}$ of the acid-extracted mucopolysaccharide is 0.06 mg/kg,[2] or approximately 4.0 mg. per 250 gm. of guinea pig.

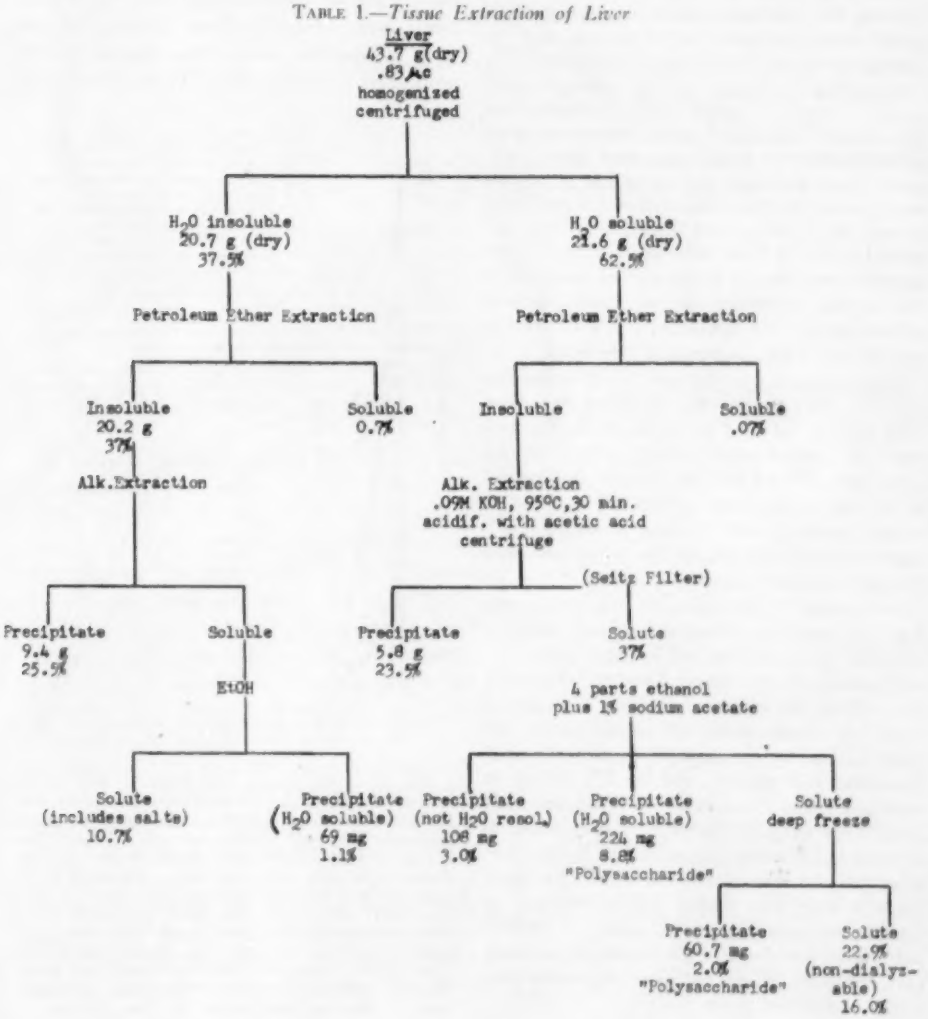
Animals.—Male and female guinea pigs, weighing 200-250 gm. at the initiation of the experiment, were given by ear vein 5 mg. of a labeled crude polysaccharide. Some animals were given daily injections for seven days. Urine was collected throughout the first day and at intervals thereafter. While in the glass urine-collection cages, the animals were fed only the cabbage supplement, instead of the usual stock diet of Purina Rabbit Pellets. At intervals, the animals were placed in sealed glass containers; O_2 was supplied by demand-regulator, and respired CO_2 was collected in CO_2-free 2 N NaOH agitated by a magnetic stirrer. By applying heat and acid to the NaOH solution, the CO_2 was collected from $Ba(OH)_2$ solution as $BaCO_3$. Similarly, acid and heat applied to the urine permitted a determination of the $C^{14}O_2$ content.

The guinea pigs were killed at various intervals up to two months. As much blood as possible was removed by cardiac puncture and the specimen

separated by centrifugation into plasma, erythrocytes, and, occasionally, buffy coat. The erythrocytes were washed three times with 0.85% NaCl. Joint washings were obtained initially by injection of 0.15 ml. of 0.85% NaCl into the right knee joint and the fluid immediately aspired. Subsequently, the same volume of distilled water, instead of saline, was used for joint washes. The three other legs were removed for histologic study. Bile was aspirated from the gall bladder. The pituitary, liver, spleen, and left adrenal were removed and weighed. Except for the pituitary, a small sample of each of these organs was taken for determination of dry weight and fat content. Whenever possible, duplicate samples of these and other tissues were taken for histologic study, autoradiography, grinding with distilled water in a glass-tube homogenizer, plating, and counting in a windowless flow counter. Urine and $BaCO_3$ samples were similarly counted. The per cent incorporation in the entire organ is given for the adrenals, liver, and spleen. To permit a comparison of these organs and other tissues as well, and also to minimize the changes resulting from the growth of the young animals, the incorporation is expressed as the per cent of injected dose per gram of dry tissue per kilogram of body weight at autopsy as calculated by the formula:

$$\frac{\text{Activity per gram tissue}}{\text{Activity of total dose}} \times \text{body weight, in gm.,} \times 100$$

TABLE 1.—*Tissue Extraction of Liver*



Serum obtained from guinea pigs, injected with the akaline-extracted polysaccharide, was reinjected into other guinea pigs. These animals were then killed at various intervals and the tissue distribution of the label determined as indicated above.

Autoradiography.—The apposition technique was used with No-Screen X-Ray Emulsion and deparaffinized microsections from acetone-formalin-fixed tissue. Comparison with dry, unfixed tissue revealed no loss of the C^{14}-labeled material by acetone-formalin fixation and by organic solvents and paraffin embedding. Decalcification procedures using 5 N HNO_3 for 18 to 24 hours resulted in loss of 75% to 80% of the C^{14}. Therefore, with decalcified sections of bone and joints, prolonged exposures up to 18 months were necessary. After staining, the tissue microsection and its autoradiograph were compared microscopically and by superimposition of photographic enlargements.

Extraction of Tissue.—At the optimum incorporation time of seven days following the intravenous injection with alkaline-extracted polysaccharide, 15 guinea pigs were killed. The spleen, liver, and right adrenal of the 15 animals were pooled; the three lots of pooled organs were ground in a refrigerated homogenizer and extracted at 4 to 6 C, as indicated in Table 1. This procedure was similar to the method employed in the alkaline extraction of the crude bacterial polysaccharide. The radioactivity of each fraction and of each wash solution was determined.

The polysaccharide fractions were hydrolyzed with 1 N HCl at 100 C for six hours, neutralized with $BaCO_3$, and used for paper chromatography with 4:1 phenol-water solvent. The resulting strips were divided in 2 cm. lengths and counted in the windowless flow counter and the relative values compared with qualitative reaction with aniline-phthalic acid reagent for sugars and with ninhydrin for amino acids.

Immunologic Procedures.—After confirming the lack of antibody formation against alkaline-extracted polysaccharide, rabbits were given several courses of heat-killed Klebsiella organisms. To establish the optimum precipitin formation, a series of concentrations of polysaccharide and rabbit antisera were quantitated by micro-Kjeldahl procedures for nitrogen and by C^{14} activity in supernatant and precipitate. In precipitin tests using tissue extracts, the extracts were added to antibody in C^{14} content comparable to the optimum range for the injected polysaccharide. Precipitin reactions were also studied qualitatively and by the ring and gel-diffusion techniques of Oudin[18] and Ouchterlony.[19] Tests for heterophile antibody were conducted before and after absorption with adrenal and kidney tissue.

Results

Loss of C^{14} from Plasma, in Respired $C^{14}O_2$ and Urine.—After intravenous injection, the labeled polysaccharides are observed in the blood plasma but not in erythrocytes or leukocytes. In the animals given the toxic acid-extraction polysaccharide, protein-rich peritoneal and retroperitoneal fluid usually accumulates during the first day and disappears within 48 hours after a single intravenous injection. In spite of such evidence of redistribution of polysaccharide or label in the body fluids during the first and second days, both alkaline- and acid-extraction polysaccharides have comparable losses from plasma and in urine and respired CO_2. When these losses

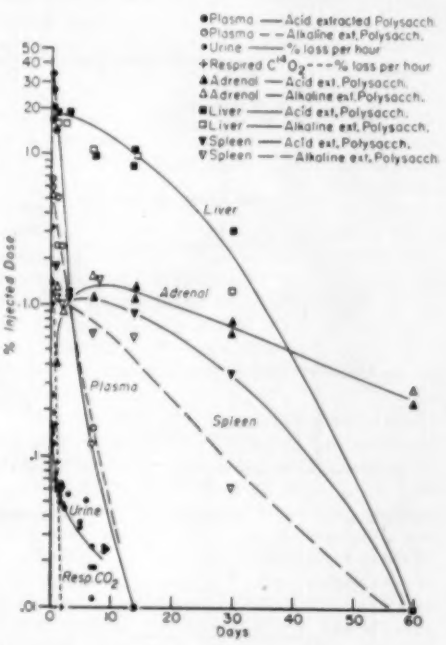


Fig. 1.—Total organ incorporation and hourly urinary and respiratory losses are similar for acid- and alkaline-extracted polysaccharides. The plasma is usually "cleared" within one week. Urinary loss is high initially, diminishes progressively, but continues at a low value for days. Respired $C^{14}O_2$ loss declines almost exponentially for the first two days, with none detectable thereafter. The liver and usually the spleen reach their maximum activities within an hour and decline thereafter. The adrenal, however, does not reach its maximum C^{14} activity for one or two weeks and maintains a relatively high value for two months.

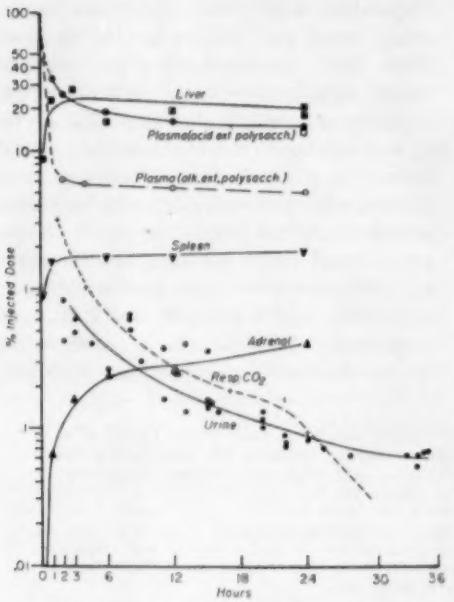


Fig. 2.—By plotting time in hours instead of days, the data for the initial period after injection are more clearly shown than in Figure 1. Plasma C^{14} values drop precipitously, leaving only 7% of alkaline-extracted and 25% of acid-extracted polysaccharide in plasma at two hours. Respired $C^{14}O_2$ and urinary losses parallel one another at first, but respired $C^{14}O_2$ suddenly disappears, while urinary loss continues. Progressive increase in adrenal C^{14} is readily noted.

are plotted, several distinct phenomena are apparent (Figs. 1 and 2): (*a*) There is a rapid decline in plasma levels, which, after the initial 16 to 24 hours, approximates an exponential "clearance" of about 5.5% of the plasma level per hour; no appreciable quantity remains in the plasma after 5 to 7 days; (*b*) An approximate exponential loss of respired $C^{14}O_2$ occurs during the first two days but none thereafter. (*c*) The rate and amount of C^{14} loss in the urine during the first 24 hours is almost identical with that of respired $C^{14}O_2$; after this period the label continues to appear at a declining rate as long as the tissues contain incorporated label, i. e., up to 1 and 2 months. During the first 24 hours 66% of the C^{14} in pooled urine samples is in nondialyzable form and at the later periods, when there is far less activity, the label remains in a nondialyzable and noncarbonate substance. By the end of seven days only 20% to 25% of the total injected polysaccharide is accounted for on the basis of urinary and CO_2 losses. Apparently, the rest is in the tissues.

Tissue Incorporation.—The label is also found very soon and in considerable quantity in the joint washings. After several days, the activity of the joint fluid on a unit (dry) weight basis often exceeds that of the plasma. Considerable variation in activity from animal to animal is expected with the technical problems of a joint-washing procedure. In addition, comparisons upon either dry weight or volumetric basis are unreliable; the dry weight of plasma and joint wash approximates 7.0 and 0.1 mg. per milliliter; 0.15 ml. injected and aspirated from the joint may considerably dilute the original content.

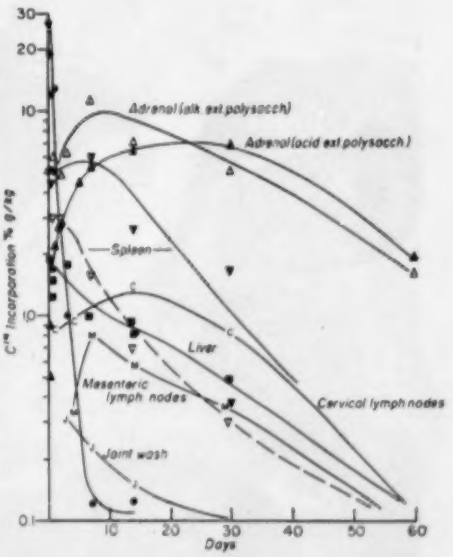
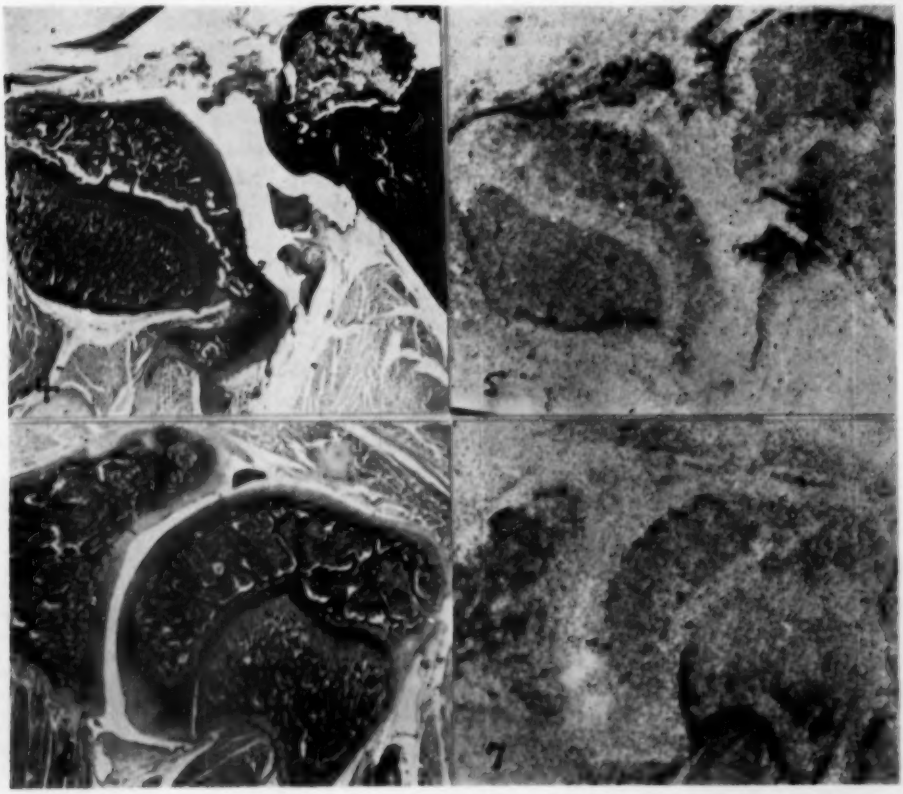


Fig. 3.—C^{14} incorporation into tissues is expressed on the unit-weight basis per cent per gram of dry tissue per kilogram (% g/kg.) instead of the total organ incorporation, as in Figures 1 and 2. Adrenal activity is the highest of any tissue. The C^{14} content of the cervical node is consistently higher than that of the mesenteric nodes. The liver activity declines exponentially. After the first day, C^{14} activity in the joint-space wash fluid is often higher than in the plasma.

The greatest quantity of C^{14} is incorporated in the liver, while significant amounts are localized in the spleen and adrenal. The liver and spleen, as well as the lung, reach their maximum levels of C^{14} within the first hour after the intravenous injection, but the adrenal incorporation rises gradually over a period of several days to attain the highest activity per unit weight of any tissue (Fig. 3). Adrenal activity remains elevated for at least two months, whereas activity declines and disappears from the liver, spleen, lung, and lymph nodes. The alkaline-extraction polysaccharide shows a higher adrenal incorporation and lower liver and spleen values than the acid-extraction product. With both polysaccharides the cervical lymph node incorporation per unit weight is also consistently higher than that of the mesenteric and tracheobronchial lymph nodes. In the 15 animals injected with alkaline-extraction polysaccharide and killed at one week, the values, in per cents per gram of dry tissue per kilogram, for cervical, tracheobronchial, and mesenteric nodes were 0.89 ± 0.30, 0.21 ± 0.20, and 0.36 ± 0.14, respectively. This variation is not explained by any difference in fat content. Relatively

Figs. 4-7.—Comparison of hematoxylin-eosin-stained section of knee joint. Figure 4, with its autoradiograph (Fig. 5), prepared by apposition technique, discloses the most intense radioactivity in the synovial lining, the meniscal, articulating, and epiphyseal cartilage being spared; moderate activity is present in the marrow. The guinea pig had received 5 mg. of C^{14}-labeled alkaline-hydrolysis polysaccharide of K. pneumoniae one week previously. In Figures 5 and 6, comparison of tissue section and autoradiograph of scapulohumeral joint from a guinea pig given a total of 35 mg. of acid-hydrolysis polysaccharide over a 10-day period and killed two weeks after the last injection shows mild radioactivity in the synovium and most intense radioactivity in the periosteal area of the humeral diaphysis.

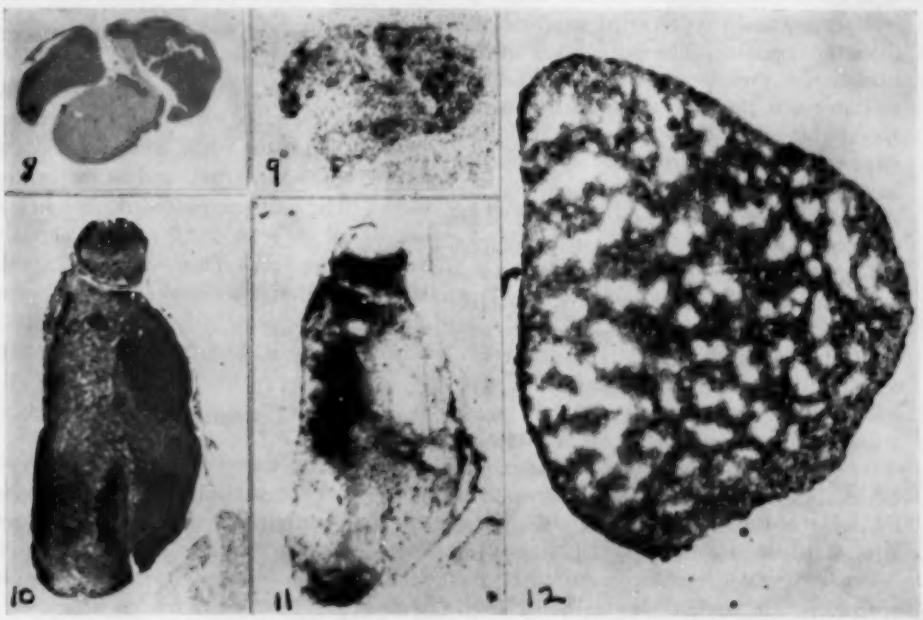


minor amounts of label are found in pituitary, salivary gland, thymus, thyroid, vitreous humor, seminal vesicle, testis, ovary, uterus, bowel, fat, skeletal muscle, myocardium, and pancreas. The label appears quite rapidly in the bile, but the nature of its chemical incorporation has not been determined. Presumably, much of the label reenters the animal from the intestinal tract, since so little appears in the feces.

Autoradiographic Findings. — The comparatively low radioactivity of tissues and the necessity of employing No-Screen X-Ray emulsion results in autoradiographs suitable for histologic localization but unsuitable for cytologic details. Activity in the joints is concentrated along the synovia, with some activity also in the adjacent areolar connective tissue (Figs. 4-7). C^{14} is also present within the protein exudate of the joints. No appreciable activity is seen within the articulating, epiphyseal, or meniscal cartilage. Bone marrow demonstrates considerable activity. The various histologic changes in the joints have been presented in previous papers with the present agents.[2,3] The C^{14} concentration is greatest in the adrenal cortex and is also seen in the splenic pulp but not in the Malpighian bodies (Fig. 12), and in the sinusoidal areas of lymph nodes but not within their follicles (Figs. 10 and 11). Radioactivity is also prominent in the delicate connective tissue just beyond the renal pelvis submucosa (Fig. 13); the cortex shows some minute areas corresponding to glomeruli. The heart valves contain a minor amount of activity, but little more than can be found in many tissues, such as endocardium, thymus, muscle, and salivary gland.

Tissue Extracts.—The extraction procedures indicated in Tables 1, 2, and 3 are

Figs. 8-12.—The pituitary (Fig. 8; × 11), as compared with its autoradiograph (Fig. 9), shows more activity in the anterior than in the posterior lobe, with apparently greater activity in some groups of cells than in others. Radioactivity in the cervical lymph node (Figs. 10 and 11; × 11) is restricted to the reticuloendothelial and sinusoidal areas and is absent in the lymphocytes. Autoradiograph of the spleen (Fig. 12; × 8) shows intense radioactivity in the pulp, with none in or immediately about the Malpighian bodies. All sections are from the same animal as that in Figures 10 and 11, with autoradiographic exposure on No-Screen X-Ray emulsion for 13 months.



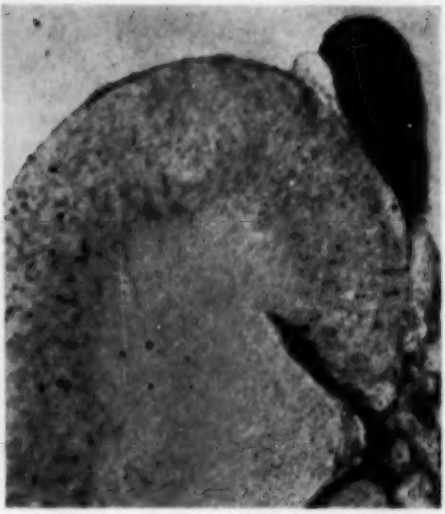


Fig. 13.—Autoradiograph of the kidney and adrenal shows marked C^{14} activity within the adrenal cortex; medulla is not present in section. There is also activity within the renal cortex, especially in the peripelvic connective tissue, the site of a cell-free protein exudate. × 7.

similar to those used for original alkaline extraction of crude polysaccharide from the bacteria, with the exception of the initial separation into water-soluble and water-insoluble fractions by centrifugation of the tissue homogenate. The distribution of the label in the various water-soluble fractions indicates that the liver and spleen have an appreciable quantity of material with the same extraction characteristics as the crude bacterial polysaccharides. However, the adrenal has a quite different distribution of the label, and little of it has the solubility characteristics of the crude bacterial polysaccharide. Very little activity is found in lipid material first extracted from the adrenal emulsion, but the fatty material liberated after alkaline extraction of the water-soluble fraction has relatively more activity. The fraction with the highest activity per milligram was obtained from the spleen, but even this tissue had less than 1% of the activity per milligram of the injected bacterial product.

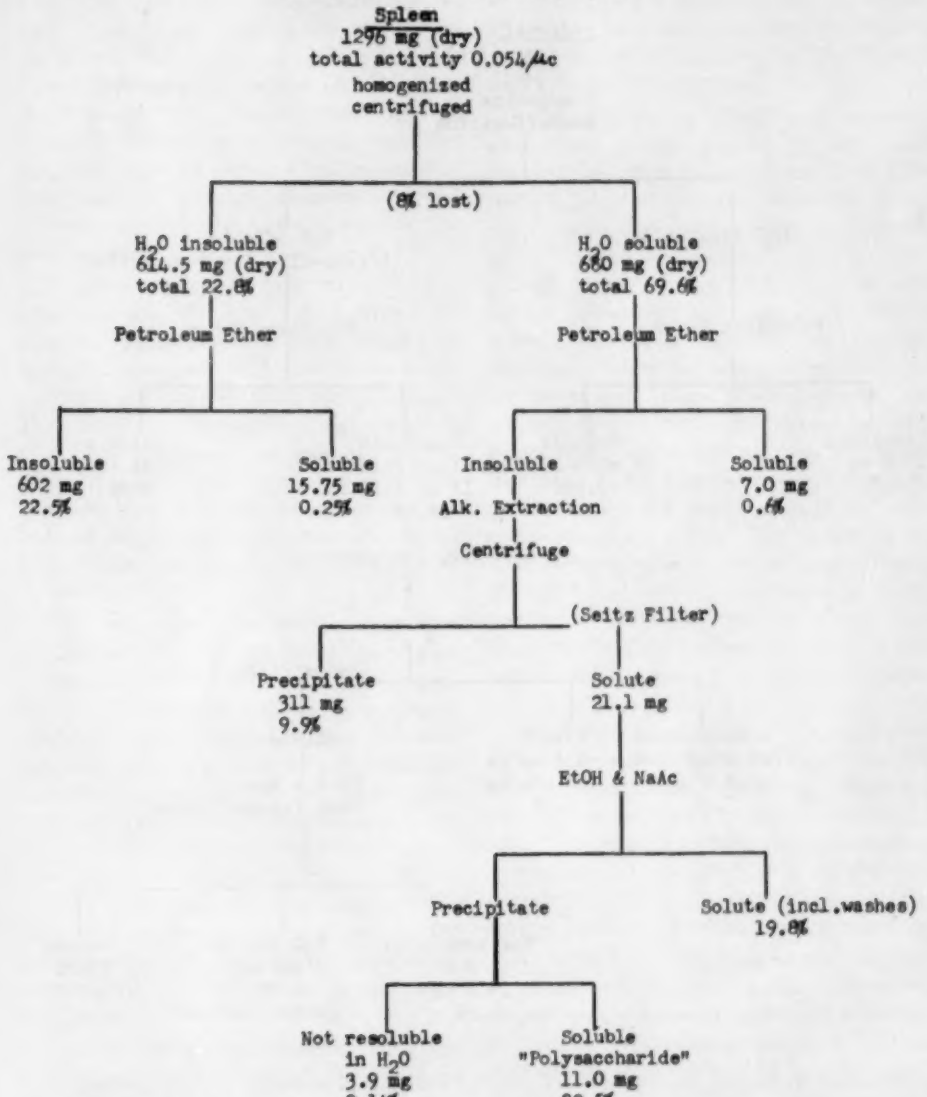
Paper chromatographic separation of the hydrolyzed polysaccharide fractions from liver discloses considerable radioactivity in the glucose regions, moderate activity in the glucuronic acid area, and variable activities for the amino acids, similar to the findings with the injected bacterial polysaccharides. Quantitative estimations of the various sugars and amino acids from tissue fractions have not been made.

Ring precipitin and qualitative precipitin tests reveal activity in the adrenal, spleen, and liver extracts. Positive results without distinct bands were obtained with the Oudin gel technique. Three definite bands appeared with the alkaline-extracted polysaccharide and anti-Klebsiella serum in the Ouchterlony preparation, but tissue extracts have been of such low antigen or hapten concentration that band formation was unsatisfactory. In the tube precipitin tests the optimum precipitin formation with alkaline-extraction polysaccharide and anti-Klebsiella serum was 20γ/ml. Using the per cent of C^{14}-labeled polysaccharide recovered in the precipitate as a standard and adding tissue extracts in isotopic amounts equal to the optimum 20γ/ml., it was observed that only 17.5% of the C^{14} activity in adrenal "polysaccharide" extract is precipitated with antibody, whereas 45% of the splenic extract, 45% of liver fraction 1, and 62.5% of liver fraction 2 are precipitated. Instead of using the water-soluble adrenal extract, an emulsion of the whole adrenal gland was mixed with antiserum and with control serum. The antisera yielded three times as much label in the insoluble material obtained by centrifugation. This suggests that the injected bacterial substance may be more difficult to separate from cellular components of adrenal than from liver or spleen.

Comment

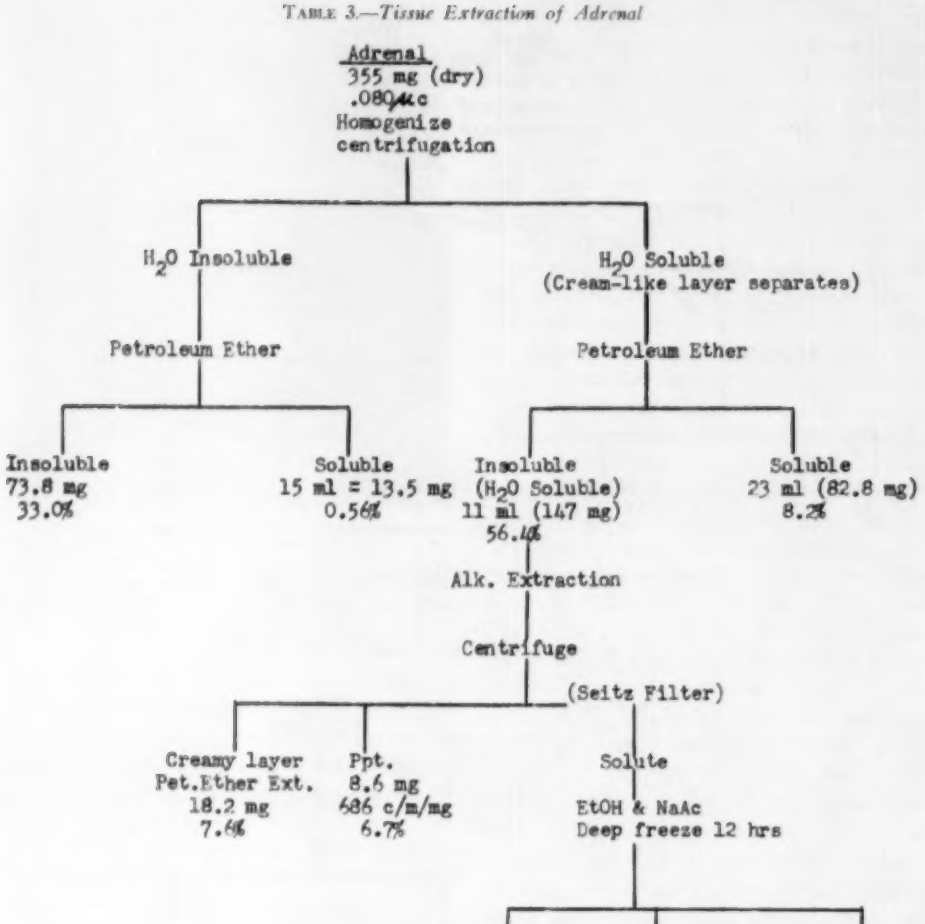
In the pathogenesis of acute arthritic lesions from the crude bacterial polysaccharide, the interrelations of host responses and host functions may be simplified by the familiar terms "direct or indirect," "local or systemic."

TABLE 2.—*Tissue Extraction of Spleen*



Correlation of synovial exudation and proliferation with the local distribution of the polysaccharide favors the direct or local action of the injurious agents. The C^{14} immediately enters the joint space from the circulating plasma and is recoverable from the joint for several weeks after the initial injection. Histologic changes closely follow the appearance of the label in the joint. Synovial cells are disrupted, and their cytoplasm apparently extrudes within a few hours. Within 6 to 24 hours globules of eosinophilic, nonmetachromatic material, apparently protein but not fibrin, appear over the synovial cells and in the joint space. By 12 to 24 hours the synovial cells have

TABLE 3.—*Tissue Extraction of Adrenal*



proliferated to several layers in thickness, often forming papillary protrusions. One week after a single 5 mg. injection of either bacterial polysaccharide, synovial proliferation and exudation are diminishing or absent.[8]

Using fluorescent antibody on histologic sections of mouse joints, Coons and coworkers [12] have observed Klebsiella capsular polysaccharide within synovial cells, joint space, and some articulating cartilage cells. From this observation and the present autoradiographic findings, it is apparent that the C^{14}-labeled polysaccharide is concentrated in the proliferating synovium. Quantitation of C^{14} uptake by synovium has not been possible by either the autoradiographic or the joint-washing techniques, but at a time when the plasma is without effect upon the autoradiographic emulsions, considerable C^{14} activity is present in the synovium and is more marked here than in the bone marrow.

The joint washings, though inaccurate, reflect a parallelism between the activity in the plasma and that in the joint space but do not determine the amount of C^{14} within the synovial cells. Future studies of the changing distribution in plasma, synovium, and joint space of the bacterial polysaccharide, in contrast to other substances, may aid in understanding the pathogenesis of the joint lesions.

Many substances of a large molecular size readily reach the joint space from the plasma. Egg albumen and horse serum soon appear in the joint fluid, and horse serum may have a greater entrance than removal.[17] The variation in permeability and exudation of various substances into body cavities and tissue spaces may be dependent not upon molecular size alone but also upon anatomical and physiological differences intervening between the blood capillaries and the body cavities.[18]

With our present knowledge, the pathogenesis of the joint lesions is speculative. Increased capillary permeability may produce the protein-rich fluid in the peritoneal cavity and retroperitoneal tissues. A similar exudation of plasma, with its polysaccharide content, could occur into the joint space, resulting in the altered metachromatic mucin and in the subsequent synovial proliferation. The immediate cause of synovial proliferation is unknown; it may represent a reparative process or a response to the concentration of foreign polysaccharide entrapped during its passage to and from the plasma.

As indicated by the autoradiographs, the localization of C^{14}-labeled polysaccharide in the spleen and lymph nodes can be attributed to reticuloendothelial activity. Cytologic detail, however, does not permit specific differentiation of large phagocytes or plasmocytes. The reason for greater incorporation in the cervical rather than in the mesenteric node is not clear. In the cervical nodes the initial edema may be more prominent and the large reticuloendothelial cells become more numerous. Through the same limitation in cytologic detail on the autoradiographs, we have not determined the relation of the basophilic particles in the hepatic cord cells to C^{14} localization.

In considering an indirect or a systemic mechanism in the genesis of the joint changes, the high concentration of C^{14} in the adrenal is of interest. While the maximum uptake by liver, spleen, and lymph nodes is within hours, the C^{14} uptake of the adrenal cortex is more gradual, reaches the highest concentration of any tissue within a few days, and is maintained in fair concentration, while becoming insignificant in the liver and spleen. Histologically, the only changes, if any, in the adrenal are little hemorrhages and minute necroses within the first day. These soon disappear, leaving no residue even while there is a high C^{14} concentration in the organ. The nature of the label incorporation in the adrenal remains obscure.[4] Far less C^{14} is extracted in "polysaccharide" fractions from the adrenal than from the liver and spleen, and less of the adrenal extract than of the liver and splenic extract is precipitated with Klebsiella antibody. The depression of C^{14} incorporation by prior treatment with cortisone but not with corticotropin, progesterone, pregnenolone, desoxycorticosterone acetate (DOCA), and 11-desoxy-17-hydroxycorticosterone (Substance S)[19] suggests that the polysaccharide or some of its derivatives are intimately involved in adrenocortical function.

A single 5 mg. injection of the alkaline-extracted polysaccharide gives a stress response with a fourfold to fivefold elevation in plasma 17-hydrocorticosteroid and a marked depletion of adrenal ascorbic acid. With two or more daily injections, the adrenal ascorbic acid is depleted for six to seven days.[20] Further studies are needed to evaluate the role of such alteration in adrenal function and ascorbic acid metabolism.

With our present knowledge, the most reasonable of many possible hypotheses for the pathogenesis of the joint lesions may be the following: A foreign mucopolysaccha-

ride by direct local effect produces a nonsuppurative exudative response in the joints; accompanying uptake of the agent in the synovium leads to proliferation of synovial cells and continued extrusion of plasma or cellular protein into the joints; concomitant alterations in adrenal cortical function and ascorbic acid metabolism may lead to the enhancement or persistence of these lesions.

Summary

The pathogenesis of the acute arthritic lesions in guinea pigs has been studied by C^{14}-labeled polysaccharide complexes of Klebsiella pneumoniae Type B. Aspects of the metabolism, tissue distribution, and nature of incorporation of the injected polysaccharides are presented.

One week after a single intravenous injection, little C^{14} remains within the plasma. Respiratory $C^{14}O_2$ loss occurs only during the first two days. C^{14} rapidly appears in the joint fluid and concentrates within the synovium. Aspiration of the joint spaces often yields material with greater C^{14} activity than the plasma.

The injected alkaline-extraction polysaccharide is retained in the liver, spleen, and adrenal. When these tissues are extracted and the recovered C^{14} polysaccharides are compared with the originally injected material, the relative haptenic properties are 45% to 62.5%, 45%, and 17.5% for the liver, spleen, and adrenal, respectively.

The pathogenesis of the joint lesions relative to these findings is discussed.

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Experimental Aberrant Lipogenesis

II. Substrate Factor

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In the previous paper of this series, it was shown that fat is formed by cells that are exposed to oleic acid or to sodium oleate in the presence of serum.[1] This occurs in vivo or, under proper conditions, in vitro. Although a phenomenon demonstrable in many tissues, as will be described in a subsequent paper, it is most easily studied in the cornea, which has a relatively uniform architecture and is readily accessible for injection experiments.

The experiments of the present paper were directed toward evaluating the specificity of the oleates as a substrate. A secondary consideration was the correlation of the oleate-induced phenomenon with the origin of fat in so-called fatty degeneration.

The technique consisted of (1) injecting the test agent into the cornea of the intact animal, (2) injecting the test agent into an excised button which was then incubated, or (3) adding the test substance to the medium in which an untreated corneal button was immersed. The buttons were incubated at 37 C in serum for 24-48 hours and occasionally longer. Although the techniques of injecting the button and inoculating the media were frequently used for the same test agent, the latter was the method of choice whenever the substance was sufficiently soluble in serum. At the end of the test period the corneal pieces were fixed in 5% formalin, sectioned in the frozen state, and stained with Sudan IV or oil Red O.

Experimental Results

The experimental results are arranged in the subsequent presentation according to the nature of the test agents used. So far as possible, the serum factor was kept constant, with variation only in the substrate. Experiments that were clearly complicated by bacterial growth were discarded.

A. Fatty Acids, Soaps, and Monoesters.

1. Oleates: Oleic acid was injected into approximately 25 corneas of living rabbits.* The amount injected was about 0.05 cc. of a 10% emulsion in saline solution. Within a matter of hours the eyes became inflamed, developed an abscess within a few days, and ultimately showed extensive scarring, vascularization, and occasionally perforation. By removing the eyes at varying time periods after the injection, it was found that, in addition to the progressive inflammatory reaction and local necrosis, fat droplets began to appear in the corneal cells adjacent to the site of injection at about 12 hours and increased for several days. This fat was variably present in all the cells (epithelium, stroma, and endothelium), depending on the area of injection. The corneal reaction was masked after the first few days by the massive invasion of inflammatory cells. However, corneal buttons that were similarly injected with oleic acid but removed from cell invasion by being placed in cellophane bags within the peritoneal cavity or beneath the skin showed a continuing process of intracellular fat formation for at least two weeks.

Similar results were obtained by injecting 10% sodium oleate (adjusted with HCl to pH 7.2) into the corneas of living rabbits. Hence the lipogenesis and toxicity are to be attributed to the oleate factor and not

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* No difference in results was found with oleic acid U. S. P. or with oleic acid purified by the method of Holman.[2]

to the acidity. (Evidence will be presented subsequently to indicate that it is not the necrotizing effect of the oleic acid which is responsible for the fat formation.)

Oleic acid and sodium oleate were also injected into corneal buttons that were then incubated in serum for varying lengths of time.† The cells immediately adjacent to the site of injection disappeared within a few hours, but intracellular fat formation occurred in the surviving adjacent cells. This was first evident in about six hours' time and increased progressively for at least seven days, which is the longest time over which such observations have been made. The cells became so filled with sudanophilic material as to be several times their normal size.‡ The only difference in results between the oleic acid and the sodium oleate experiments was that the lipogenesis in the former was somewhat more limited to the area immediately adjacent to the necrotic tissue.

Incubation of untreated corneal buttons in a serum-oleate medium was the most productive of uniform results. Observations have now been made on several hundred such buttons. The optimum concentration has been found to be 5-10 mg. of sodium oleate per milliliter of serum. The incubation of each button was carried out in 3 ml. of serum, with a change of medium each day. Lipogenesis was seen first at about six hours, increasing progressively for at least a week. All the cells (epithelium, stroma, and endothelium, when present) showed the fat formation without evidence of necrosis.

Calcium oleate (1% suspension in 0.9% sodium chloride solution) induced a qualitatively similar lipogenesis when injected into the cornea of living rabbits. This, however, was less marked and less constant than when similar amounts of oleic acid or sodium oleate were injected. It also caused less inflammation.

Methyl oleate injected into the cornea of living rabbits or into corneal buttons that were subsequently incubated showed an active lipogenesis and necrosis identical with that of oleic acid and sodium oleate.

A series of observations were made on polysorbate 80 U. S. P. (Tween 80), the oleate ester of sorbitol. This was injected into the corneas of rabbits or inoculated into the incubation media as in the foregoing experiments with sodium oleate. The results obtained in approximately 20 experiments with polysorbate 80 were identical with those when sodium oleate was used. Thus, on injection into the cornea of living rabbits or into corneal buttons which were subsequently incubated, polysorbate 80 induced an intense necrosis with an active lipogenesis in the adjacent surviving cells. Similarly, addition of polysorbate 80 to serum in the incubation experiments resulted in lipogenesis in corneal buttons. The optimal concentration of polysorbate 80 was 10 mg/ml. of serum. Less than this yielded progressively less fat formation, while more than this was toxic to the cells. Incubation of corneal buttons with polysorbate 80 in Tyrode's solution, either with or without an aminoacetic acid (glycine) buffer and with or without glycerin, resulted in no appreciable lipogenesis.

2. Nonoleates: No lipogenesis was induced when any of the following organic acids were injected into the corneas of living animals in molar concentrations comparable to that of the oleic acid. Several of these acids were as necrotizing and as pyogenic as oleic acid. Specifically, negative results were obtained with acetic acid, butyric acid, *n*-caproic acid, pelargonic acid, undecylenic acid, stearic acid, palmitic acid, and arachidic acid. On the other hand, positive lipogenesis was obtained with unpurified samples of elaidic acid and linolenic acid, but these two may well have had oleic acid present as well.

No lipogenesis was induced by incubation of corneal buttons in serum which was forti-

† It is most convenient to make the injections in the corneas after enucleation of the eye but before the buttons or pieces are excised. In this way several buttons or pieces may be obtained from each cornea.

‡ See Figure 1 in previous article.[1]

fied with sodium acetate, butyrate, purified elaidate, stearate, or palmitate. Indeed, the only soap, other than oleate, yielding significant lipogenesis was sodium linoleate, and the purity of this was questionable.

Negative results were also obtained with glycerol monostearate, methyl stearate, ethyl linoleate, polyoxyethylene sorbitan monostearate (Tween 60), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monolaurate (Tween 20).

It thus appears that of all the fatty acids, soaps, or esters that have been tried, oleic acid with its soaps and esters is alone capable of inducing lipogenesis as we have defined it. The possible exception to this is linolenic acid.

This lipogenesis is not dependent on a necrotizing property or on any feature which we have been able to identify other than what is inherent in the oleate molecule.

B. Neutral Lipids.—Fat extracted with ether from the rabbit omentum and injected as a suspension into the rabbit cornea was detectable as sudanophilic globules for months. These were for some time extracellular and showed, for the first week at least, none of the intracellular globules which we have associated with lipogenesis. Nor did lipogenesis occur in corneal buttons injected with neutral fat and incubated in serum or in normal buttons incubated in a serum which had been fortified with neutral fat and cholesterol. Similarly, no lipogenesis occurred with injection of olive oil, triolein, or paraffin into the corneas of living animals. If, however, these neutral fats were saponified beforehand and then injected, a vigorous lipogenesis occurred.

Neutral lipids thus induce no lipogenesis comparable to that which occurs when oleates are injected. It is true that eventually the fat is taken up by some cells in the cornea, but the rate with which this occurs, the absence of participation by the corneal fibrocytes, the later macrophage response, and the associated formation of birefringent crystals are sufficiently different to distinguish this from the phenomenon of lipogenesis which occurs with the injection of oleate compounds or, as will be immediately described, with the injection of tissue components.

C. Phospholipids.—A vigorous lipogenesis occurred with the injection of lecithin into the corneas of living animals.§ The obvious explanation for this is that the oleate of lecithin is liberated and induces an oleate reaction. But the oleate in neutral fat, triolein, or olive oil cannot be liberated by the cornea.

D. Tissue Components. — 1. Blood: Whole rabbit blood or washed red blood cells injected into the rabbit cornea in vivo resulted in considerable fat formation in the adjacent fibrocytes and the appearance of fat in macrophages. Unlike the situation when oleates were injected, however, this was not apparent during the first week, and when it did occur, in the second to the fifth week, it was not accompanied by any necrosis or inflammation in the tissue.

Rabbit plasma injected into the cornea failed to induce any fat formation. On the other hand, normal human plasma injected into the rabbit cornea resulted in a variable, but occasionally considerable, fat formation that was evident by the second week. This was not regularly greater when serum was used from patients with xanthomatoses than with normal serum.

Corneal buttons incubated in serum occasionally showed a small amount of fat formation even without the addition of oleates. This did not occur if the lipids were first removed from the serum, whereas it was increased if the animals supplying the serum were heparinized within the hour prior to drawing the blood. Similarly, the one human serum sample that gave the greatest amount of fat formation was from a patient who had xanthomatosis and had received heparin one hour prior to drawing the blood sample. On the other hand, adding heparin

§ Lipogenesis occurred equally with commercial egg lecithin and with lecithin purified according to the method of Pangborn.[3,4]

to serum in vitro had no effect on the fat formation.‖

Attempts to study lipogenesis in corneal buttons injected with plasma of cholesterol-fed rabbits were inconclusive because the large amount of free fat in the injected material masked the new fat formation in the cells.

From the foregoing observations, based on approximately 35 separate experiments, it was concluded that blood could itself induce active lipogenesis in the tissues. The most lipogenic component was in the red blood cells, but the delay in appearance of the fat, in comparison with the oleate experiments, suggested that the substrate first had to be liberated from the blood cells. Serum also had not infrequently a lipogenic factor. This, however, was slight and variable. That which was present was in the lipid factor of serum and was enhanced by treatment with heparin prior to obtaining the serum. The few observations that were made on normal human serum suggested that this was more lipogenic than rabbit serum, but serum from patients with xanthomatoses was not more lipogenic than normal unless the patients were previously heparinized. These latter observations merit further study.

2. Corneal Tissue: Corneal tissue which had been ground up in sand and subsequently suspended in saline solution was injected into the cornea of living rabbits. Only rarely did any fat form in the adjacent cells, and this was never more than a trace.

This experiment was done by way of a control to test whether corneal damage per se could be responsible for lipogenesis. It was concluded that damage to corneas incident to the injection was not responsible for significant lipogenesis and, incidentally, that collagen, which makes up most of the cornea, was not a substrate for lipogenesis.

3. Liver Tissue: The following observations on liver are based on approximately 35 experiments. They include injection into the cornea of emulsion of whole liver, lipid extracts of liver, and fat-free liver.

Injection of whole liver into corneas in vivo resulted in a prompt and vigorous lipogenesis in the adjacent fibrocytes, which, as in the case of the oleate injections, was strikingly evident within the first few days. Unlike the comparable oleate experiments, however, there was little or no necrosis or inflammation of the host tissue. The lipogenesis occurred with liver tissue which was first washed free of blood or first treated with potassium cyanide or sodium fluoride. Lipogenesis similarly occurred when whole liver emulsion was injected into corneal buttons that were then incubated in serum. On the other hand, little or no lipogenesis occurred in corneal buttons that were incubated in serum to which the liver emulsion was added.

Liver tissue was extracted for a week in ether, and the extract, after evaporation of the ether, was taken up as a 10% suspension in saline solution. This was then injected into the corneas in vivo and into corneas that were subsequently incubated in serum. In all cases the extract induced a considerable lipogenesis and some necrosis.

Liver tissue remaining after the ether extraction also induced an abundant and prompt lipogenesis when injected into the cornea but without appreciable necrosis. The lipogenesis was, however, abolished if the liver tissue was further extracted with methanol and chloroform,¶ prior to the injection.

From the foregoing it was concluded that liver tissue contained a lipogenic component comparable to the oleate factor. This was present in the ether-extractable fraction and in a fraction in which the responsible constituent appeared to be a lipoprotein. This latter differs from simple oleates in not being appreciably necrotizing. The results are compatible with the thesis that it is the oleate constituent of tissue which is respon-

‖ The observations on heparinized plasma were prompted by the finding that injection of heparin results in a lipolysis of the blood fats (Anfinsen et al.[5]).

¶ As advised by Folch and Lees for extraction of lipoproteins.[6]

sible for tissue-induced lipogenesis, but the binding of this oleate to other constituents prevents its necrotizing property.

4. Brain Tissue: The results of injecting brain tissue and extracts of brain into the cornea were similar to those of injecting liver and liver extracts. They have been described in detail and illustrated elsewhere,[7] along with observations on foam-cell production, so that a summary only will be given here. The number of experiments were about 35, divided among injection of whole-brain suspension, lipid extract, and "fat-free residue."

Whole-brain tissue injected into the cornea of the living rabbit resulted in abundant lipogenesis in the adjacent fibrocytes without significant necrosis or inflammation. Similarly, brain lipid extracted for a week in ether and resuspended in water resulted in lipogenesis without necrosis when injected into the cornea. Extraction with methyl alcohol and chloroform (Folch's Fraction A, believed to contain fats, phospholipids, and proteolipids) and the ether extract of this (Folch's Fraction B, believed to contain fats and phospholipids) both induced lipogenesis when injected into corneas in vivo and in corneal buttons. The so-called "fat-free" residue resulting from the ether extraction produced considerable lipogenesis when injected into corneas but not in residues that had been further extracted with methyl alcohol and chloroform.

On the other hand, emulsion of whole brain or of brain lipid failed to induce lipogenesis when simply added to the serum of the incubation medium.

From the foregoing it was concluded that brain tissue contains a substrate analogous to, if not identical with, the oleate factor. This component in brain is in both the ether-soluble fraction and the proteolipid. Defatted brain material will support lipogenesis only as long as it has at least one of these compounds. The lipogenic factor in brain, like that in liver, differs from oleic acid or sodium oleate in that it is nonnecrotizing. It seems entirely possible that the responsible substance in brain is an oleate radical maintained in a bound form.

Comment

The tentative thesis developed from the foregoing experiments is as follows: The oleate radical is a specific prerequisite for the formation of sudanophilic fat by the corneal cells. Moreover, an oleate-containing substance will induce lipogenesis only when it is present in an available form. Thus it is abundant with oleic acid, oleate soaps, oleate monoesters, or lecithin, but not in the form of triolein or other neutral fats. Tissue extracts vary in their ability to support lipogenesis. Thus extracts of corneal tissue, composed largely of collagen, have no significant lipogenic factor; blood has a variable amount, whereas liver tissue, brain tissue, and certainly many others (kidney, spleen) can support lipogenesis abundantly. The lipogenic factor in these tissues is both in the proteolipids and in the ether-soluble component. It seems entirely reasonable to assume that it is the oleates in these tissues that are responsible for the lipogenesis.

Insofar as the changes resulting from injecting emulsified tissue into a foreign host are comparable to degeneration in situ, one may apply the foregoing thesis to the origin of fat in so-called fatty degeneration and atherosclerosis. It thus seems likely that fat associated with fatty degeneration arises in considerable measure at least by a synthesis of fat from an oleate-containing substrate. This differs from the usual concept of liberation of masked fat or ingestion of preformed fat brought to the cells. Further observations on the dynamic aspects of this lipogenesis will be presented in the subsequent paper, dealing with the tissue factor in the phenomenon.

To the best of our knowledge, these observations and conclusions are essentially new. However, pertinent observations bearing on it have been made from various sources. Thus, the fat formation which has been repetitively observed in tissue cultures may be attributable to the presence of

oleates. Simms [8] found that vacuoles in some of his cultures were due to soaps retained on the glassware after incomplete rinsing. Simm's "lipfanogen" [9] probably contained oleates, for we, too, have found a substrate factor in the lipid phase of human plasma. On the other hand, the specificity of oleates in this process was unexpected and unexplained. Needless to say, further observations on this process are needed at the tissue-culture level.

Conclusions

1. Experimental lipogenesis (synthesis of neutral fat) depends on the presence of oleic acid, some oleate-containing compound, or some lipid derivative of tissue which is believed to contain oleates.

2. Lipogenesis does not occur with neutral fats, native or otherwise, unless they are hydrolyzed previously.

3. Fatty acids other than oleic acid cannot be substituted for oleic acid.

4. It is suggested that oleic acid or the oleate molecule is essential for the production of fat in fatty degeneration and atherosclerosis.

243 Charles St. (14).

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Leukemoid Blood and Leukocytes in Treatment of Radiation Lethality

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Congdon et al.[1] obtained a 10%-70% survival of lethally irradiated mice injected with whole blood showing extreme granulocytosis (leukemoid blood). They showed further that the effectiveness of leukemoid blood was associated with the formed elements but not the plasma. These observations are particularly pertinent in view of the evidence indicating that infection, a major cause of death for lethally irradiated mammals, is correlated with granulocytopenia (review by Cronkite and Brecher[2]). Our aim in these experiments was to find out how leukemoid blood causes recovery in x-irradiated mice. Leukocytes from sources other than leukemoid blood were also tested for recovery activity in lethally irradiated mice.

Materials and Methods

Freshly drawn whole blood, or its formed elements, was used for postirradiation treatments. In most experiments, the blood was obtained from BALB/c JAX mice bearing a transplantable squamous-cell carcinoma (A280). This carcinoma, which induces a leukemoid response, developed from a spontaneous skin tumor after additional painting with 20-methylcholanthrene.* In other experiments blood was obtained from man, rabbits, and rats.

The blood was withdrawn from the heart of anesthetized (pentobarbital [Nembutal] sodium) laboratory animals into syringes containing heparin. When whole blood injections were to be used, a leukocyte count was made and, if necessary, saline added to adjust the preparation to the desired white cell concentration.

Separation of the formed elements was carried out by several procedures. Fibrinogen (6% in saline), an erythrocyte-agglomerating agent, was used for isolation of leukocytes. The technique used was essentially the one described by Skoog and Beck.[3] Platelets were obtained by differential centrifugation of whole blood according to a procedure of Cronkite et al.[4] Erythrocytes were obtained by centrifuging whole blood at 150×g for eight minutes, leaving many leukocytes and platelets in the supernatant. The sedimented erythrocytes were removed, resuspended in saline, and the suspension centrifuged at 80×g for eight minutes. This procedure, repeated five to seven times, gradually reduced the leukocyte and platelet concentration to a minimum. Each of the formed elements was suspended in saline for injection.

The preparations were tested in mice that had received a single lethal dose of radiation generated by a 250 kv. General Electric Maxitron x-ray machine operating at 30 ma. The beam was filtered through 0.5 mm. Cu plus 1.0 mm. Al, giving a dose rate in air of about 80 r/min. The half-value layer was 1.32 mm. Cu. Male and female mice 3-4 months old were irradiated in groups of 12 in a continuously revolving, partitioned cage made of Lucite placed 64.0 cm. from the x-ray tube. Within four hours after irradiation, about 0.5 ml. of the preparation to be tested was injected into the tail vein. The effectiveness of each preparation was judged by the number of mice surviving 30 days or more after irradiation, and, in some experiments, by the mean death time.

Results

Injection of Whole Leukemoid Blood.—A single intravenous postirradiation injection of whole leukemoid blood promoted up to 100% survival of lethally irradiated BALB/c mice (Table 1A). The results suggest a direct correlation between the number of leukocytes injected and the number of mice surviving 30 days.

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* The A280 tumor originated in Dr. W. R. Bryan's laboratory at the National Cancer Institute, Bethesda, Md.

TABLE 1.—*Effect of Single Injection of Whole Leukemoid Blood on Thirty-Day Survival of Irradiated (750 r) BALB/c Mice*

	Treatment	No. of Leukocytes Injected (× 10^6)	30-Day Survival, %	No. of Mice
A	Intravenous injection, postirradiation	1.0	0	7
		10.0	9	12
		25.0	63	18
		56.0	84	19
		78.0	60	10
		100.0	100	20
	Control	None	0	70
B*	Intravenous injection, preirradiation	87.0	0	12
	Intravenous injection, postirradiation	87.0	75	4
	Control	None	0	12
C*	Intravenous injection, postirradiation	192.0	100	11
	Intraperitoneal injection, postirradiation	192.0	75	12
	Control	None	0	7

* Injections made from one homogeneous batch of leukemoid blood.

The results of one experiment show that whole leukemoid blood injected immediately before irradiation was ineffective (Table 1B).

Leukemoid blood was effective when given either intraperitoneally or intravenously (Table 1C). Leukemoid blood from one batch was used for the two routes of injection. Results from these and similar experiments show, however, that leukemoid blood was less effective intraperitoneally than intravenously.

The injection of whole leukemoid blood containing 132.0×10^6 leukocytes into CAF_1, C_3H X $101F_1$, and LAF_1 mice exposed to 900 r and into RF mice exposed to 800 r did not increase the percentage of 30-day survival. Other studies, with irradiated CAF_1 (BALB/c X A) mice, showed, however, that the mean death time was increased by the injection of whole leukemoid blood containing several hundred million leukocytes (Table 2A,B). Furthermore, when the x-ray dose was reduced from 900 to 750 r, all the controls died; however, 4 of the 11 leukemoid blood-injected CAF_1 mice survived 30 days. The mean death time was significantly increased from 11.7, for controls, to 23.0 days, for the injected mice (Table 2C).

A single injection of bone marrow from leukemoid mice (BALB/c) into irradiated CAF_1 mice enhanced the 30-day survival (Table 2B).

Injection of Leukocyte Suspensions.—When lethally irradiated BALB/c mice were given a single postirradiation injection of leukocytes from leukemoid blood, the 30-day survival varied from 0 to 100%, depending on the number of white blood cells injected. The results shown in Table 3A were obtained from one experiment in which all the injected mice received fractions of a single pooled leukocyte preparation. Results presented in Table 3B show the survival obtained in several experiments with different preparations of leukocyte suspensions. Thirty-day survival was variable, but with

TABLE 2.—*Effect of Single Intravenous Injection of Leukemoid Blood on Thirty-Day Survival and Mean Death Time of Irradiated CAF_1 Mice*

	Postirradiation Injection	No. of Leukocytes Injected (× 10^6)		30-Day Survival, %	X-Ray Dose, r	Mean Death Time, Days	No. of Mice
A	Leukemoid blood	Exper.	435.0	0	900	16.0	6
	None	Cont.	0	0	900	9.1	6
B	Leukemoid blood	Exper.	500.0	0	900	15.5	4
	None	Cont.	0	0	900	9.6	3
	Bone marrow	Cont.*	--	100	900	----	5
C	Leukemoid blood	Exper.	300.0	36	750	23.0	11
	None	Cont.	0	0	750	11.7	12

* These control animals received bone marrow (one femur per mouse) of mice from which the leukemoid blood was obtained.

TABLE 3.—*Effects of Single Intravenous Injection of Leukocyte, Erythrocyte, or Platelet Suspensions from Leukemoid Blood on the Thirty-Day Survival of Irradiated (750 r) BALB/c Mice*

	Cells Injected	No. of Cells Injected ($\times 10^6$)	30-Day Survival, %	No. of Mice
A	Leukocytes	1.0	9	11
		10.0	40	10
		30.0	50	10
		50.0	90	12
		127.0	100	8
		140.0	100	12
	Controls	None	0	10
B	Leukocytes	10.0	0	10
		30.0	50	10
		51.0	81	17
		83.0	57	7
		100.0	71	11
		123.0	100	16
	Controls	None	0	80
C	Erythrocytes	1600.0	0	6
		165.0	0	12
		5.0	0	7
		0.9	0	16
	Controls	None	0	18
D	Platelets	410.0	0	6
		258.0	0	12
		150.0	0	5
		100.0	0	9
		62.0	0	6
	Controls	None	0	19

some indication of a direct relation to the number of leukocytes injected. It is also seen that equal numbers of leukocytes from different batches of blood did not result in the same percentage survival (compare 10×10^6 leukocytes in sections A and B of Table 3).

The leukocyte preparations from leukemoid blood contained both erythrocytes and platelets as contaminants. There were from 5.0- to 50.0×10^6 erythrocytes and 50.0- to 150.0×10^6 platelets in each injection. Injection of these or greater quantities of erythrocytes or platelets from leukemoid blood failed to give any 30-day survival (Table 3C,D). Two or more irradiated mice were injected with leukocytes of the blood from which erythrocytes or platelets were obtained; at least 50% of these controls survived 30 days.

TABLE 4.—*Effect of a Single Intravenous Injection of Heterologous Leukocyte Suspensions on Thirty-Day Survival of Irradiated (750 r) BALB/c Mice*

Source of Leukocytes	No. of Leukocytes Injected ($\times 10^6$)	30-Day Survival, %	No. of Mice
Man, myeloid leukemia	165.0	0	29
Man, stem-cell leukemia	165.0	0	5
Rabbit, normal	100.0	0	10
Rat, normal	50.0	0	6
Control	None	0	24

Leukocytes from other species were tested for their ability to bring about recovery in BALB/c mice. Leukocyte suspensions from normal Sprague-Dawley rats, from rabbits, and from man with either myeloid or stem-cell leukemia proved to be ineffective when injected into irradiated BALB/c mice (Table 4).

Morphology of Leukemoid Blood (Figs. 1,2).—The striking feature of leukemoid blood is its extreme granulocytosis. In the

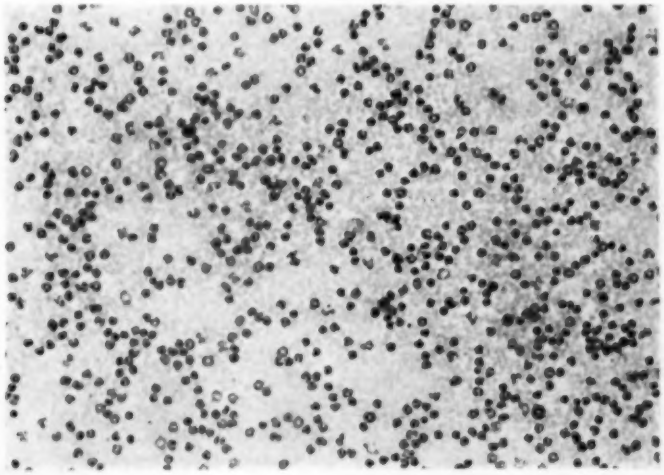

Fig. 1. — Smear of leukemoid blood. Note the extreme granulocytosis. Wright-Giemsa stain; × 145.

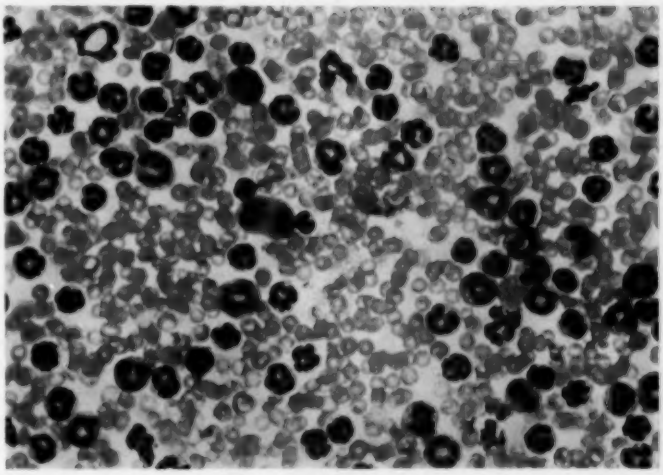

Fig. 2.—Enlarged section of Figure 1. Note the mitotic figure (late anaphase) at left center of the photograph. At the top and to the left, a large mononuclear cell can be seen. Wright-Giemsa stain; × 445.

peripheral blood of a tumor-bearing mouse having a white cell count of about 200,000/cu. mm., 70%-90% of the white blood cells are polymorphonuclear leukocytes and granulocytes with a ring-shaped nucleus. Myelocytes and metamyelocytes are present in variable numbers. A small, variable number of cells, possibly blast cells, are relatively large and have a basophilic cytoplasm and a spherical nucleus, containing finely divided chromatin. Monocytes are present. The absolute numbers of reticulocytes and lymphocytes are increased. Infrequently, a dividing cell is observed. In an effort to standardize the quality of leukocytes, we used only leukemoid blood with a total leukocyte count of at least 200,000/cu. mm.

Comment

The present results show that leukocytes from isologous leukemoid blood cause recovery of lethally irradiated BALB/c mice. One outstanding feature of leukemoid blood is the preponderance of mature granulocytes. Perhaps these cells combat the infection that is a major factor in irradiation death. If the animal escapes death from infection, it may then restore blood cells from reticular tissue that has survived irradiation. It does not seem likely, however, that a single injection of leukemoid blood or its leukocytes would maintain a sufficient concentration of granulocytes over a period long enough to control infection.

Another explanation for the recovery action of these leukocytes involves the release of a humoral substance from the leukocytes that stimulates hematopoiesis. If such a substance exists, it is inactivated by subjection of the leukocytes to sonic vibration or alternate freezing and thawing, as preliminary results from this laboratory have indicated.

The presence of immature leukocytes and mitotic figures in leukemoid blood suggests a recovery action similar to that of bone marrow, since infused marrow cells have been shown to implant, proliferate, and function hematopoietically in irradiated recipients.[5-8] Our results show that leukemoid blood, like bone marrow, was ineffective when injected before irradiation and was effective intraperitoneally as well as intravenously. Also, like bone marrow,[9] the number of injected leukocytes of leukemoid blood was directly correlated with the survival after lethal total-body irradiation. Congdon et al.[1] observed that injection of leukemoid blood effects recovery of hematopoietic tissue and the peripheral leukocyte count similarly to bone marrow treatment. Lorenz and his associates[10] showed that lethally irradiated mice of one strain survive 30 days or more if given bone marrow

from another strain of mice (homologous therapy). We have found that bone marrow from normal BALB/c mice enhances the 30-day survival of x-irradiated CAF_1 and LAF_1 mice and that bone marrow from leukemoid BALB/c mice is also effective in x-irradiated CAF_1 mice. If, therefore, the actions of leukemoid blood and bone marrow are similar, the former should be effective homologously. The leukemoid blood was not effective homologously in lethally irradiated LAF_1, C_3H X $101F_1$, and RF mice; but it is possible that insufficient numbers of leukemoid blood leukocytes were injected. This was apparently the case when BALB/c leukemoid blood was given to its F_1 hybrid (CAF_1). The injection of leukemoid blood containing 132.0×10^6 leukocytes did not enhance the 30-day survival or mean death time of x-irradiated (900 r) CAF_1 mice, but injection of 435.0×10^6 leukocytes significantly increased the mean death time of CAF_1 mice from 9.5 to 16.0 days. In addition, the injection of 300.0×10^6 leukocytes into CAF_1 mice exposed to 750 r enhanced the 30-day survival and significantly increased the mean death time, although the surviving mice were in poor condition. Congdon et al.[1] reported that 3 of 10 lethally irradiated DBA mice given leukemoid blood survived more than 30 days, although the bone marrow did not regenerate.

According to some of the parameters studied, leukemoid blood apparently acts like bone marrow in causing recovery of lethally irradiated mice. When leukemoid blood is used as a recovery agent, it is conceivable that only the granulopoietic elements repopulate the irradiated recipient's bone marrow and spleen from precursors in the leukemoid blood. If this hypothesis is correct, erythrocyte and platelet recovery should come from the recipient's tissues, and not those of the donor. The observation that leukemoid blood was more effective in CAF_1 mice at the lower radiation dose (750 r) might support this concept.

Leukocytes from leukemic patients and from normal rats and rabbits were tested for recovery activity in BALB/c mice but were ineffective. Species larger than mice were chosen as donors primarily because they provided large numbers of leukocytes. Leukocytes from patients with myeloid or stem-cell leukemia were used because of the quality, as well as quantity, of the white blood cells. Attempts to obtain large numbers of leukocytes (i. e., 50.0×10^6) from normal mice have not been entirely successful. In one experiment, two of eight lethally irradiated BALB/c mice that received about 50.0×10^6 leukocytes from normal BALB/c mice have survived more than 150 days. These results, however, are inconclusive.

Summary

It has been shown that leukocytes of leukemoid blood from BALB/c mice cause recovery of lethally irradiated BALB/c mice. The data indicate that the number of mice surviving 30 days or more is directly proportional to the number of leukocytes injected. One hundred per cent survival is usually obtained with 100.0×10^6 or more leukocytes. Neither erythrocytes nor platelets of leukemoid blood enhance the 30-day survival.

The data suggest that the mode of action of leukemoid blood in the treatment of radiation lethality is similar to that of bone marrow.

In this study, heterologous leukocytes from normal rats and rabbits and from man with leukemia proved ineffective in the treatment of irradiation injury in mice.

Miss Bonnie Anderson helped in the preliminary work on leukocyte separation.

Biology Division, Oak Ridge National Laboratory, P. O. Box Y.

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Renal Failure and Other Unusual Manifestations in Sickle-Cell Trait

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Sickle-cell trait is commonly thought of as a benign hematologic disorder occurring in approximately 6%-9% of the Negro population. Since the initial report of Bauer and Fisher,[1] other investigators have attempted to emphasize the occasional serious and variable nature of this condition.[2-6] We have recently seen one such case, in which some previously undescribed findings have been noted. Because of the apparent rarity of these cases, opportunity will be taken to report this case from the standpoint of the clinical, hematologic, and autopsy findings. We should like to reemphasize the occasional fatal sequelae of this disease.

Report of a Case

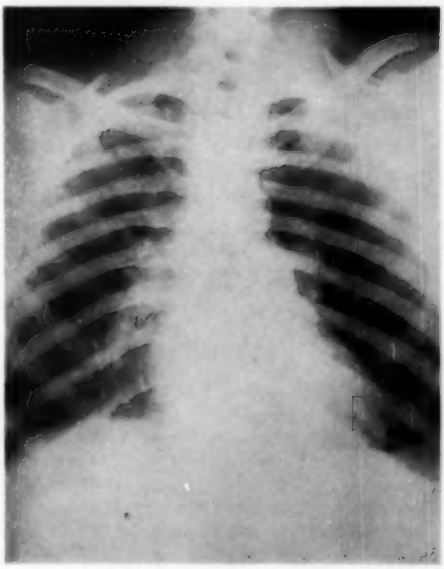
A 35-year-old Negro man was first admitted to the Albert Einstein Medical Center, Southern Division, on Oct. 10, 1953, with sudden, severe precordial oppression associated with shortness of breath. The past medical history and systemic review were noncontributory except for an episode of generalized joint pains and fever in 1945. Physical examination revealed B. P. 135/80, P 96, R 16, and T 103 F. There was no pallor, icterus, adenopathy, hepatosplenomegaly, or purpura. The remainder of the physical examination was essentially negative. The blood showed RBC 4,200,000 hemoglobin 12.5 gm. per 100 cc., WBC 14,500 per cubic millimeter, with 7% bands, 76% segmented forms, 12% lymphocytes, 4% monocytes, and 1% eosinophils. The BUN was 8 mg. per 100 ml. of blood.

Repeated electrocardiograms revealed nonspecific changes, with temporary T-wave inversion. A supine chest x-ray was reported as normal. The patient improved spontaneously and was discharged two and one-half weeks after admission.

Fourteen months later, on Dec. 16, 1954, he was readmitted because of left-sided chest pain associated with fever, chills, and shortness of breath. Examination at this time revealed B. P. 110/75, P 80, R 24, and T 103 F. There was no pallor, icterus, purpura, or glossitis. Generalized cervical adenopathy and hepatomegaly were found. The spleen was not palpable, although bilateral costovertebral-angle tenderness was present. The remainder of the physical examination was essentially normal.

The blood count showed RBC 4,280,000, hemoglobin 14.1 gm. per 100 cc., WBC 7,450 per cubic millimeter, with 4% bands, 68% segmented forms, 24% lymphocytes, 3% monocytes, and 1% basophils. The blood smear showed slight anisocytosis, poikilocytosis, and hypochromia and minimal polychromatophilia. A moderate number of orthochromatic target cells were seen. The sickling preparation was positive; ESR 57 mm. in one hour, BUN 88 mg. per 100 ml. of blood, Na 141 mEq., K 4.5 mEq., Cl 97 mEq., and CO_2 21.9 vol. %. The urine showed 4+ albumin with 1 to 2

Fig. 1.—The trabecular pattern of all the ribs is prominent and coarsened.



Submitted for publication July 31, 1956.

From the Department of Pathology, Albert Einstein Medical Center, Southern Division.

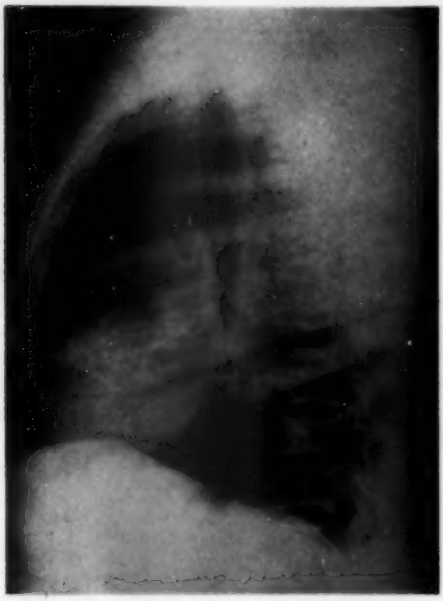


Fig. 2.—The vertebrae show flattening and broadening of the bodies with biconcave deformities of the superior and inferior margins.

RBC and 10 to 15 WBC per high-power field. Chest x-ray showed slight cardiac enlargement. The trabecular pattern of all the ribs was prominent and coarsened. X-rays of vertebrae showed flattening and broadening of the bodies with biconcave deformities of the superior and inferior margins and central demineralization. Throughout the bony pelvis and femoral heads there were poorly defined areas of increased density associated with some areas of increased radiolucency. The composite bone findings were interpreted as showing changes compatible with sickle-cell anemia (Figs. 1 and 2).

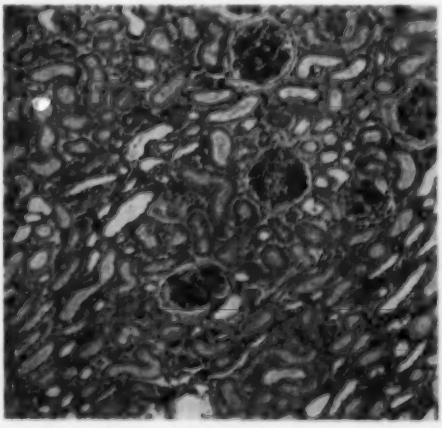
The hospital course was marked by continual fever, vomiting, and diarrhea. The blood count on Dec. 17 showed RBC 4,400,000, hemoglobin 14.1 gm. per 100 cc., and WBC 15,700 per cubic millimeter, with 8 nucleated RBC per 100 WBC. A serum bilirubin of 1.25 mg. per 100 cc. (0.4% direct, 0.85% indirect) was obtained. Scleral icterus was now noted for the first time. On Dec. 21 the RBC count was 4,090,000, hemoglobin 13.3 gm. per 100 cc., and WBC count 26,900 per cubic millimeter, with 19 nucleated RBC per 100 WBC. Scleral icterus became more marked. The patient died on Dec. 21, 1954, five days after admission, in uremia.

The pertinent gross autopsy findings were as follows: The heart weighed 300 gm. and showed no evidence of recent or healed myocardial infarction. There were moderate bilateral pulmonary edema and congestion of the lungs. The liver weighed 1700 gm. and was moderately congested and firm. The spleen, although markedly congested, weighed but 80 gm. and revealed increased trabecular markings and thickening of the capsule. The kidneys were congested, each weighing 200 gm. and showing focal pyelonephritic scars.

Microscopic sections revealed the sinusoids of the liver and spleen to be markedly engorged with sickle cells. The blood vessels of the heart, lungs, kidney, gastrointestinal tract, and bone marrow were focally engorged with sickle cells. The kidneys showed minimal, focal, chronic pyelonephritis. The glomeruli were engorged with sickle cells, and many of the glomeruli were diminished in size despite this engorgement. There was a clear amorphous fluid in Bowman's capsule about these glomeruli (Figs. 3 and 4).

There were focal siderofibrosis and increased trabecular fibrosis of the spleen. Special stains for iron pigment proved the presence of hemosiderin in the spleen (Fig. 5). A normoblastic hyperplasia of the bone marrow was present. Microscopic study of the vertebral column revealed thinning and focal erosion of the bony trabeculae with marked diminution of osteoblastic activity. These microscopic observations correlate with the observed x-ray findings (Fig. 6).

Fig. 3.—A representative section of the kidney shows preserved structure and congestion mainly confined to the glomeruli.



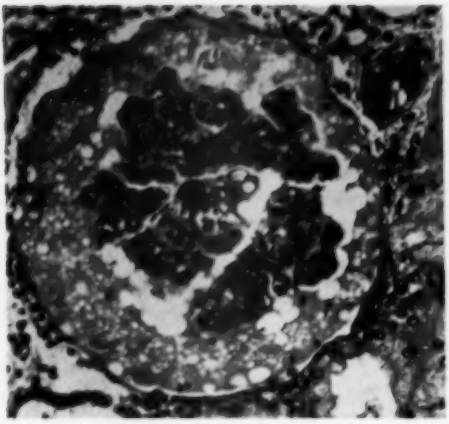


Fig. 4.—The glomerulus is markedly engorged with sickle cells and a collection of amorphous fluid is present in Bowman's space.

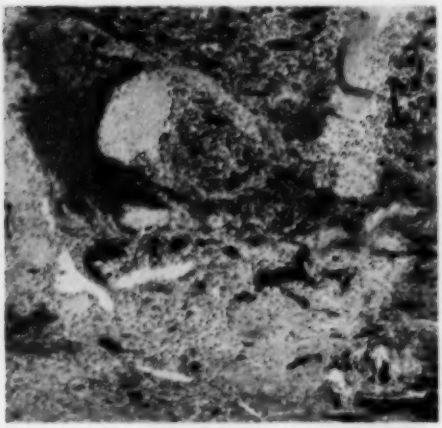


Fig. 5.—The spleen shows increased trabecular fibrosis and focal siderofibrosis. Marked engorgement with sickle cells is noted.

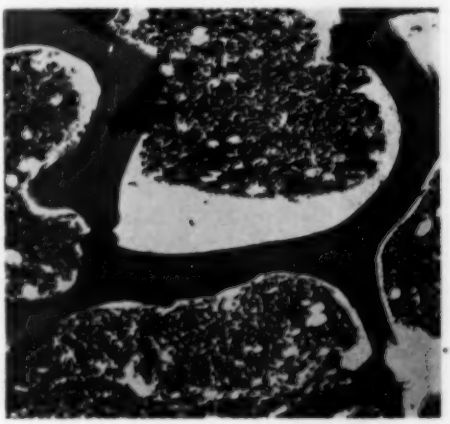
Comment

The presence of the normal red cell count and hemoglobin associated with a positive sickling preparation undoubtedly meant that our patient had the sickle-cell trait. He did show several of the stigmata of sickle-cell anemia or hemoglobin S-C disease, i. e., bone changes by x-ray, a relatively small spleen, and infiltration of all organs with sickle cells. Terminally, scleral icterus, increased bilirubin, and nucleated red cells in the peripheral smear, despite a normal blood count, were noted.

Focal siderofibrosis and increased trabecular fibrosis of the spleen were present. The importance of blood transfusions in the production of hemosiderosis has recently been stressed.[7] Since our patient never received any blood transfusions, we must assume that siderofibrosis resulted from splenic infarction secondary to the hemolytic process which was present. This also accounted for the bone changes seen on x-ray film and the small spleen. Several of the symptoms of sickle-cell anemia were also present, i. e., bone, chest, and joint pains with fever. Whether the hemolytic process was constant or intermittent is difficult to state. The paucity of symptoms over many years favors the assumption of intermittent hemolytic episodes, whereas the roentgen changes and hemosiderosis are in favor of a chronic process.

Recent reports have stressed the importance of low oxygen tension inducing splenic infarcts in patients with sickle-cell trait.[8-10] These have been reported in military personnel flying at altitudes of 10,000 to 15,000 ft. in nonpressurized cabins. Unfortunately, samples of their peripheral blood smears were not available at the time of their initial symptoms. It remains to be determined whether these patients had sickle cells in their peripheral blood or whether

Fig. 6.—The bony trabeculae are thinned, with marked diminution of osteoblastic activity. The marrow is hypercellular.



the sickling was restricted to their organs. It must be stressed that our patient never showed sickle cells in the peripheral blood smear despite evidence of increased hemolysis. This would favor the sickling phenomenon occurring in the various organs rather than in the peripheral blood.

The production of azotemia is most interesting. There was little histologic evidence for pyelonephritis being the cause of the patient's terminal uremia. Glomerular occlusion by sickle cells seems to be the more likely explanation. This could account for the bilateral costovertebral-angle tenderness which was present. Uremia is not a prominent finding in sickle-cell anemia, and the benign nature of the renal lesion in the trait has been stressed.[11-13] In our patient the trait was apparently severe enough to be considered the principal cause of renal failure.

Of all similar previously reported patients with sickle-cell trait, only one developed evidence of red cell destruction accompanied with a decrease in the red cell count and hemoglobin. The spleen of this patient was markedly enlarged; so we must presume he had a persistent, rather than an intermittent, hemolytic process. Our patient was never anemic; yet he had hematologic evidence of increased red cell destruction, i. e., bilirubinemia and peripheral normoblasts; apparently his bone marrow could compensate for this hemolytic process.

Analysis of the 23 previously (Table) reported cases of sickle-cell trait with unusual manifestations[1-6] revealed the patients' ages to range from 14 to 80 years. No significant sex difference was observed. All save two were Negroes. The white patients were of Italian and Greek extraction. Three were severely anemic and should be considered as having sickle-cell anemia. Reticulocytosis was noted in three, one of whom had an intermittent anemia with normal blood values during remissions. Peripheral normoblasts were found in three; bilirubinemia, in four (following blood transfusion in one). Sickle cells were never seen in any of the peripheral blood smears. A positive sickling preparation was recorded in nine (two of whom were white). Bone changes compatible with sickle-cell anemia were found in two. Three patients had marked splenomegaly. Very small spleens were observed in three, two with a severe anemia. Siderofibrosis of the spleen was found in two. Cerebral infarcts were found in four; renal and pulmonary infarcts, in one, and splenic and renal in-

*Analyses of Previously Reported Cases**

Author	Case No.	RBC	Hb/Gm.	Retic.	Nuc. RBC per 100 WBC	Serum Bilirubin	Sickling	Abnormal Hb	X-Ray Bones	Spleen, Gm.
Bauer and Fisher	1	5.15	98%							550
	2									620
	3									25
	4	1.92	35%							25
	5	0.32	1.2		5		+			
	6	3.48	54%							2.5
Canby et al.	7	4.74	12.0	9%	5	I.I.† 44	+			830
Thompson et al.	8	4.96	15.0							110
Green and Conley	9	5.47	12.0			I.I. 10-15	+	+(?)	+	
	10	5.64	11.0	2%		Tot. 3.0 Dir. 1.2	+		+	
	11	5.05	12.3	9-30%	50	Tot. 1.6 Dir. 0.7	+			+
		1.57	3.8							
Ende et al.	13						+	S.A.; 0.9% F		
	14	5.2	14.0				+	34.8% S 65.2% A 0.4% F		
	15	5.6	15.0				+			200
	16	4.6	14.0				+	S.A.; 0.5% F		
Pratt-Thomas and Switzer	17		12.0							
	18		11.0							
	19		78%							
	20		16.0							
	22	4.90	13.0							
	23	4.70	12.5							
Tellem et al.	24	4.28	14.1		8-19	Tot. 1.25	+		+	80

* For Case 12 under Green and Conley and Case 21 under Pratt-Thomas and Switzer; no laboratory values were available.
† I.I.—icteric index.

farcts, in another. Paper electrophoresis of the hemoglobins were performed on the blood of three patients and gave the characteristic findings of sickle-cell trait. One was reported as showing an "abnormal hemoglobin." Unfortunately, electrophoresis of hemoglobin was not done in our patient. One can assume that the pattern of sickle-cell trait, or the combination of hemoglobin S with another abnormal variant, would have been found.

Summary

A case of sickle-cell trait with unusual clinical, hematologic, and pathologic findings is reported. A review of similar cases in the previous literature is presented. Sickle-cell trait, per se, as a cause of renal failure is emphasized.

L. W. Diggs, M. D., Director of Department of Medical Laboratories, University of Tennessee, College of Medicine, reviewed the slides on this patient. Harold J. Isard, M. D., and staff interpreted the roentgen findings.

Department of Pathology, Albert Einstein Medical Center, Southern Division (47).

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Effects of Colchicine and Serratia Marcescens Polysaccharide on Protoplasmic Viscosity

II. Influence of Reduced Protoplasmic Viscosity on Ehrlich Ascites Tumor Growth

EDWIN T. NISHIMURA, M.D., and JOSEPH H. BAUM, B.S., Chicago

Introduction

In our previous report[1] evidence was presented to show that Serratia marcescens polysaccharide, like colchicine, inhibits the mitotic division of Ehrlich ascites tumor cells in vivo. The reduction in protoplasmic viscosity of tumor cells and the arrest of mitoses following the intraperitoneal administration of these substances into mice bearing the tumor were considered causally related.

In dividing cells, the normal completion of mitosis probably depends on certain optimal states of viscosity of the protoplasmic colloid.[2,3] Accordingly, the development of spindle cannot proceed normally if the protoplasmic viscosity is lowered below a critical level which is incompatible with mitotic gelation.[4-7]

This report presents the results of the effects of repeated administrations of Serratia marcescens polysaccharide and of colchicine U. S. P. on the growth pattern of Ehrlich ascites carcinoma in male Strong A mice.

Materials and Methods

Ehrlich ascites carcinoma* which had been maintained for two years by weekly transfers in Strong A mice was used for this study. Mice bearing tumors of 10 to 14 days were used as donors. They were killed by cervical dislocation, and the ascitic-fluid samples were aseptically aspirated with sterile 22-gauge needles and tuberculin syringes. From each sample, 0.2 ml. was retained in the syringe for viability and nucleated cell counts, and the rest was transferred into a sterile test tube. Using a small aliquot from the syringe, the viability of tumor cells was estimated by Schrek's technique[8] with 0.05% eosin in Tyrode solution. From another aliquot in the syringe, the ascitic-fluid suspension was diluted 1:200 with an erythrocyte-diluting pipette, using a diluent consisting of 0.85% NaCl solution to fill about one-half volume of the pipette, followed by a 1% HCl solution for the remainder. By this technique, coagulation of the protein-rich ascitic fluid was prevented. The suspension was thoroughly mixed, and the nucleated cell count† was made, using a Spencer Bright-Line hemacytometer.‡ After correction for cell viability, the aseptic suspension from the test tube was diluted with sterile 0.85% NaCl solution so that the final preparation constituted 10,000,000 viable tumor cells per milliliter.

Male Strong A mice between the ages of 3 to 6 months were used in this study. They were quartered in an air-conditioned room and given unrestricted amounts of Rockland Mouse Pellets and water. Each was given an inoculum of 2,000,000 viable tumor cells intraperitoneally. The growth curve for these mice was determined in the manner described by Klein and Révész[9] and Révész and Klein.[10] Briefly, the method allowed the determination of the total number of tumor cells in each animal after the initial tumor inoculation and at various moments of the active growth period of the tumor. For the estimation of the total tumor cells in a given animal, the necessary procedures followed after killing the mice at 24- and 48-hour intervals are as follows:

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From the Department of Pathology, Northwestern University Medical School.

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* Originally obtained from Dr. Robert Schrek, Veterans Administration Hospital, Hines, Ill.

† 90% were tumor cells in mice with 10- to 14-day-old tumors.

‡ American Optical Company, Buffalo.

(*a*) Determination of the number of nucleated cells per milliliter of the ascitic fluid

(*b*) Viability count of tumor cells using 0.05% eosin in Tyrode solution

(*c*) Differential cell count of the ascitic-fluid suspension to estimate the extent of leukocytic exudation

(*d*) Cell-packed volume determination of the ascitic-fluid suspension

(*e*) Determination of the total ascitic-fluid volume, using the sulfobromophthalein (Bromsulphthalein) dilution method.

All procedures were followed as previously described,[9,10] but slight modifications were made by us in two tests. Since, in this investigation, toxic substances were administered to determine their effects on the growth of the tumor, we considered the estimations of the tumor-cell viability and the degree of leukocytic infiltration into the ascitic fluid to be important. For counting the viable tumor cells, Schrek's technique was found quite adequate and easily performed. For the purpose of cellular differentiation we preferred the use of methyl green-pyronin Y stain to the Papanicolaou stain, described by Klein and Révész. By this method, the cytoplasm of the tumor cells was stained dark pink to lavender, while the nuclei appeared greenish blue. The cytoplasm of polymorphonuclear leukocytes remained pale and almost colorless, but the nuclei appeared greenish blue, as in the tumor cells. Very few lymphocytes appeared in the peritoneal fluid; however, when present, they offered no difficulty in identification because of their characteristic nuclei. The staining procedure for the determination of total tumor cells consisted of smearing the ascitic-fluid samples from the animals that were killed on clean microscopic slides. The slide preparations were then dried in air and stained, without fixation, using the methyl green-pyronin Y method described by Taft.[11]

For estimation of the cell-packed volume of the ascitic-fluid suspensions, a microhematocrit method § was used. The technique was standardized so that three capillary tubes were employed for each sample and the average value was taken for the growth data.

Experiments and Results

The growth of Ehrlich ascites carcinoma in male Strong A mice with an initial inoculum of 2,000,000 tumor cells was reported earlier.[1] The pattern during the cube-root growth phase was found to be essentially the same as that described by Klein and Révész[9] for their 1,800,000-cell inoculum. This growth curve was therefore used in our present investigation for the purpose of evaluating the effects of repeated intraperitoneal injections of Serratia marcescens polysaccharide (MP5LL4), colchicine, or 0.85% NaCl solution (solvent control) on the tumor development.

I. *Effect of MP5LL4 Polysaccharide Administration.*—A. The mice were divided into two groups 24 hours after intraperitoneal inoculations of 2,000,000 tumor cells. One group, of 10 mice, was started on three intraperitoneal injections of 30γ of MP5LL4 polysaccharide ‖ daily. In these mice the first injection was given at 8 a.m., the second at 1 p.m., and the third at 5 to 6 p.m. To parallel this experiment, a second group, of 11 mice, was used as a control, and each animal was given three injections of 0.33 ml. of 0.85% NaCl solution at the times indicated above.

Pairs of mice from each group were killed 48 hours after the initial tumor inoculation and at intervals of 24 hours thereafter. For the 144-hour sample, three control mice receiving injections of isotonic saline solution were killed instead of the usual pair. From each animal a total count of viable tumor cells was made, as outlined in the preceding section.

B. In another experiment the mice were also separated into two groups after the initial tumor inoculation. A group of 14 mice were started on repeated intraperitoneal injections of 20γ of MP5LL4 polysaccharide ¶ every five hours around the clock. The first dose, as before, was given 24 hours after the tumor inoculation. Eleven control mice bearing the tumor were given 0.2 ml. of 0.85% NaCl solution intraperitoneally every five hours, as in the mice above. Two animals from each group were killed daily for five consecutive days. The first set was killed three hours after the initial dose of the polysaccharide. The sam-

§ International Hemacrit Centrifuge, Model PR-2, International Equipment Co., Boston.

‖ From a stock solution containing 100γ per milliliter of 0.85% NaCl solution.

¶ From a stock solution containing 100γ per milliliter in 0.85% NaCl solution.

ples for the sixth determination were taken 168 hours, and for the seventh, 216 hours, following the tumor inoculation. The total tumor-cell determinations were made as before.

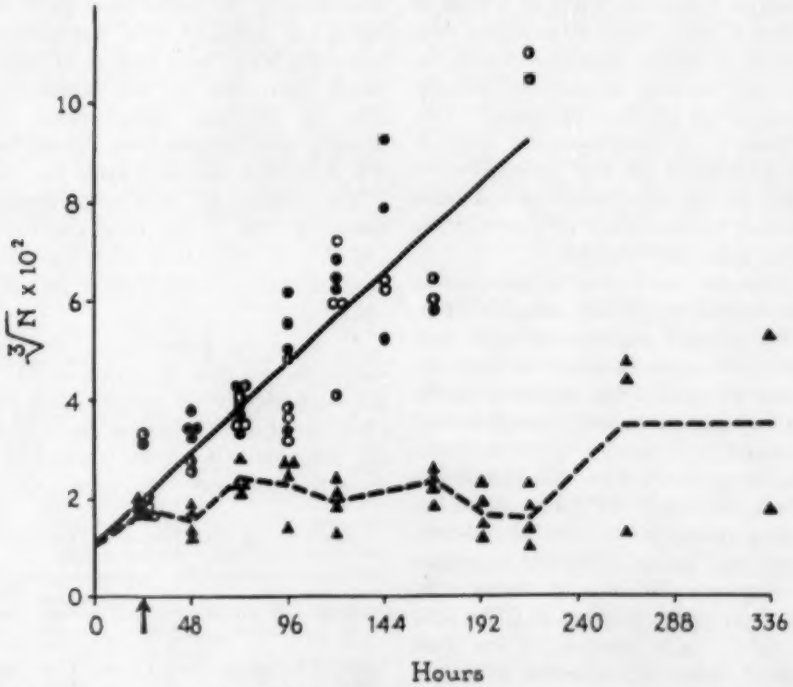
Repeated injections of the polysaccharide in the two preceding experiments show no effect on the viability of tumor cells, since the values were within the average range of untreated or saline-treated controls of 95% to 98%. Multiple polysaccharide injections, however, caused the percentage of leukocytes to increase from an over-all mean of 36% of the total nucleated cells in the ascitic fluid, for the saline-treated controls, to a mean of 70%, for the group receiving three polysaccharide injections daily, and of 71%, for the animals receiving injections every five hours.

The results of the tumor growth in mice which received MP5LL4 polysaccharide or saline show no apparent difference in the pattern of neoplastic growth for these animals (Figure). The line drawn through the mean values of the scattergram is essentially the same as the curve for the untreated tumor cells described in our previous work.

II. *The Effect of Repeated Colchicine Administration.*—Since 0.85% NaCl solution, used to dissolve the MP5LL4 polysaccharide and colchicine U. S. P., was shown to have no effect on the tumor growth, further controls using this solvent were not examined.

For this portion of our study, 36 mice with a 24-hour growth of tumor from an initial inoculum of 2,000,000 cells were given daily intraperitoneal colchicine injections of 1.0γ per gram of body weight.#

From a stock solution containing 80γ/ml. in 0.85% NaCl solution.



Scattergram showing the effects of MP5LL4 polysaccharide, colchicine, and saline administration on the growth of Ehrlich ascites tumor. Half-solid circles represent MP5LL4 polysaccharide, 20γ every five hours; circles, MP5LL4 polysaccharide, 30γ three times daily; triangles, colchicine, 1γ per gram of body weight per day; solid circles, 0.85% NaCl three or five times daily. All injections were given intraperitoneally.

Three mice were killed two hours after the first dose of colchicine. The remainder were killed in groups of four at intervals shown in the Figure, except that three and two mice were used for the periods representing 264 and 336 hours, respectively. The total cell-count determination was made for each animal in the manner already discussed.

The results presented in the Figure show a marked inhibition of tumor growth for at least 216 hours with daily intraperitoneal injections of colchicine. This is in sharp contrast to the absence of any effect on the growth with MP5LL4 polysaccharide. After 216 hours, a rise of probable significance in the growth curve is noted for the colchicine-treated group.

III. *Production of Antibody to MP5LL4 Polysaccharide and Development of Resistance to Colchicine.*—Since both MP5LL4 polysaccharide and colchicine were effective in causing a temporary arrest of mitosis at metaphase in this tumor,[1] experiments were designed to determine what factors were responsible for blocking the continued mitosis-inhibiting action of these substances. Two possibilities were considered—the elaboration of antibody by the host against the test substance and the development of resistance or tolerance by the tumor cells against the substance under investigation.

A. Serratia marcescens polysaccharide has been shown to possess antigenic properties.[12-15] Several experiments were performed to determine whether an antibody was produced against this substance by the host after one or more intraperitoneal injections.

1. In our previous work, the intraperitoneal administration of MP5LL4 polysaccharide into mice with Ehrlich ascites carcinoma was shown effectively to reduce the protoplasmic viscosity of tumor cells from a mean viscosity value of 0.97 ± 0.02 to 0.66 ± 0.04 for a duration of less than five hours.[1] Since the reduction in protoplasmic viscosity was shown to precede the development of metaphase arrest, and since we, among others, believe that these phenomena are causally related, the effect of repeated administrations of the polysaccharide on the viscosity of tumor cells was determined. One mouse bearing a tumor of 10 days' growth was given 30γ of MP5LL4 polysaccharide intraperitoneally. After 24 hours, a test dose of 30γ of MP5LL4 polysaccharide was given intraperitoneally. Fifteen minutes later a sample was aspirated from the abdominal cavity, and, after making a base count of polar cells, a standard centrifugation test was made for the determination of the viscosity value (VV), as previously described.[1,16]

In another experiment three mice with tumor were given 20γ of the polysaccharide. Three days later a test dose of 20γ was administered into the abdominal cavity of each. Fifteen minutes after this dose the VV of the aspirated tumor sample was determined for each mouse.

In the last experiment of this series, two mice bearing the tumor were given 20γ of MP5LL4 polysaccharide intraperitoneally every five hours for a total of 21 injections. Seven days after the first injection a test dose of 20γ was administered. Fifteen minutes later samples were drawn, and the VV determinations were made for each.

The results of these experiments are shown in Table 1. The mean viscosity value (MVV) of 1.00 ± 0.02 after the final test dose indicates no reduction in protoplasmic viscosity.

2. Since the preceding in vivo experiments showed that the action of MP5LL4 polysaccharide on the tumor cells is blocked after one or more injections, an in vitro test was designed to determine whether or not a protective antibody-like agent developed

TABLE 1.—*In Vivo Immunity Test Against MP5LL4 Polysaccharide*

Animal No.	Dose/Injection, γ	No. Injections Prior to Test Dose	Time Lapse Between First and Test Dose, Days	Viscosity Value (VV)
IM-1	30	1	1	0.99
IM-2	20	1	3	1.00
IM-3	20	1	3	1.05
IM-4	20	1	3	1.03
R-3	20	21	7	0.94
R-4	20	21	7	0.96
			Mean =	1.00
			S.E. =	±0.02

TABLE 2.—*In Vitro Immunity Studies with MP5LL4 Polysaccharide*

Animal No.	Dose/Injection, γ	No. Injections Prior to Test Dose	Time Lapse Between First and Test Dose, Days	Amt. Used for Test, γ	VV	
					Before Washing	After Washing
SPS-8	30	2	1	1	1.00	0.52
S-4	20	21	5	10	0.85	0.63
S-5	20	21	5	10	0.93	0.78
R-4	20	21	7	30	0.96	0.61
				Mean =	0.94	0.64
				S. E. =	±0.04	±0.06

against the polysaccharide and made its appearance in the ascitic fluid.

One mouse with 10 days' growth was given two intraperitoneal injections of MP5LL4 polysaccharide of 30γ each, with an interval of 24 hours. In another group three mice with 10-day-old tumors were given 20γ of the polysaccharide every five hours for a total of 21 injections. After variable periods following the last injection, the mice were killed and the ascitic-fluid suspensions were drawn for the tests. Each 1 ml. sample was first diluted with an equal volume of 0.85% NaCl solution and after mixing thoroughly, the samples were centrifuged slowly at approximately 1000 rpm on a standard clinical centrifuge for five minutes. The supernatant fluid was discarded, and the cells were resuspended and washed twice in 5 ml. of isotonic saline using slow centrifugation. The original volume of 1.0 ml. was then reconstituted with 0.85% saline solution. After making the base counts of polar cells, 1γ to 30γ of MP5LL4 polysaccharide was added (Table 2) to the washed-cell suspensions, and the mixtures were centrifuged by the high-speed technique for the determination of viscosity values. The results shown in Table 2 suggest the presence of a protective antibody in the ascitic fluid which is readily removed from the tumor cells by washing with isotonic saline solution.

In a further modification of the in vitro test, six mice with tumors of 7-10 days' growth were "immunized" against MP5LL4 polysaccharide by administering intraperitoneally one or two injections of 20γ of the polysaccharide. Three days after the last dose of MP5LL4 polysaccharide, the animals were killed and their ascitic fluid was aspirated. The suspensions were pooled and centrifuged at a low speed for 10 minutes, and the supernatant fluid was separated and kept in the refrigerator until ready for use. The packed tumor cells were discarded. For controls, six mice with tumors, but without previous polysaccharide injections, were killed and the ascitic-fluid supernate was prepared in a similar manner and refrigerated as before. Four untreated mice bearing tumors of 10 days' growth were killed and their ascitic fluid was aspirated. Each tumor sample was then separated into two aliquots of 1.0 ml. each. To one aliquot, 1.0 ml. of the treated ascitic fluid was added. To the other aliquot, 1.0 ml. of untreated (control) supernatant ascitic fluid was introduced. For testing the effect of the polysaccharide on the cells, 1.0γ of MP5LL4 polysaccharide was added to each mixture. After standing for 15 minutes, the mixtures were centrifuged by the standard method at 10,000g for 30 minutes at 0 C. The viscosity values were determined as before. The results in Table 3 clearly show that the "immune" ascitic fluid effectively neutralizes the viscosity-reducing activity of MP5LL4 polysaccharide.

TABLE 3.—*Protective Antibody Against MP5LL4 Polysaccharide: in Vitro Test*

Animal No.	Amount of MP5LL4 per Test, γ	VV	
		Untreated Tumor Cells plus "Unimmunized" Ascitic Fluid	Untreated Tumor Cells plus "Immune" Ascitic Fluid
IM-0	1	0.76	1.02
IM-5	1	0.66	1.02
IM-6	1	0.62	1.00
IM-7	1	0.80	0.98
	Mean =	0.71	1.01
	S.E. =	±0.03	±0.01

TABLE 4.—*Effects of Colchicine on Tumor Cells Previously Treated with Multiple Injections of the Drug*

Animal No.	Total* Dose Colchicine γ	VV After Test Dose of Colchicine			MI After Test Dose of Colchicine	
		0	15 Min.	17 Hr.	0	17 Hr.
CR 1	72	0.91	0.91	1.00	2.0	2.2
CR 2	72	0.98	0.98	0.98	1.5	1.0
CR 3	72	1.04	1.04	0.97	2.5	2.0
CR 4	72	0.99	0.95	1.03	1.8	3.6
CR 5	96	0.93	0.96	0.97	0.9	1.7
Mean		0.97	0.97	0.98	1.7	2.1

* Given over a period of five to six days. Does not include the final test dose of 1.0 γ/gm. wt.

B. The effect of repeated colchicine injections in mice with Ehrlich ascites tumor was shown to depress the growth curve (Figure), although none of the 36 mice showed a complete absence of tumor cells in the samples of peritoneal fluid. At 264 and 336 hours a definite increase in the total number of tumor cells was noted for three animals. We considered this increase to be significant and believed that it was related to increased resistance or tolerance of the tumor cells to colchichine at the dosage maintained in this experiment. The loss of effectiveness of colchicine by repeated injections of the alkaloid in mice with Ehrlich ascites tumor was reported by others.[17,18] It might be expected that a test dose administered to animals with tumor cells which have been made resistant to the drug by a series of colchicine injections would have little or no viscosity-reducing effect. According to this view, the mitotic index should not increase in pretreated animals as a result of the metaphase arrest, since the cell viscosity necessarily remains unaltered. To test this concept experimentally, five mice with 7- to 10-day tumors were given intraperitoneal injections of colchicine daily for five or six days with a dose of 1.0γ per gram of body weight per day. Twenty-four hours after the last injection, samples of ascitic fluid were drawn, and a final test dose in the amount previously given was administered. Ascitic-fluid samples were then taken at 15 minutes and 17 hours after the test dose. The viscosity values were determined and the smears made as before for each sample. The smears were stained by the HCl-acetic acid-orcein staining technique [1] for the determination of the mitotic index.

The results tabulated in Table 4 show that there is no effect on the viscosity values at 15 minutes or at 17 hours. Furthermore, no significant effect on the mean mitotic index is noted at 17 hours. Samples from Animal CR 4, however, show a slight rise in the mitotic index, from 1.8 to 3.6. With this one exception, no elevation of the mitotic index is encountered. These results support the contention that the development of mitotic inhibition by colchicine in Ehrlich ascites tumor is related to, and probably dependent upon, the reduction of viscosity in the protoplasm of the tumor cells.[1]

Comment

The biological effects of Serratia marcescens polysaccharide have been studied extensively because of the ability of the substance to produce hemorrhage and necrosis in some experimental tumors of animals. For reasons yet unknown, this substance injures, and sometimes completely destroys, certain malignant tumors of mesenchymal origin when administered to mice bearing these neoplasms.[19-21] Malignant epithelial tumors, on the other hand, seem less affected by the action of the polysaccharide.[20,21] The tumor-necrotizing activity of this complex substance has been attributed to the phenomenon of the Shwartzman reaction.[22,23] Studying the polysaccharide from another approach, Heilbrunn and Wilson [5] showed that it inhibits mitotic division in eggs of the sea annelid Chaetopterus by reducing the protoplasmic viscosity. They believe that

interference with mitotic gelation, which, in turn, prevents spindle formation, results in mitotic arrest. In our previous report, a preparation of Serratia marcescens polysaccharide labeled MP5LL4 was obtained through the courtesy of Dr. Hugh J. Creech, of the Institute for Cancer Research of Philadelphia, and this material was examined for its ability to inhibit mitoses of Ehrlich ascites tumor. The substance was found effectively to reduce the protoplasmic viscosity of Ehrlich ascites tumor cells. Two intraperitoneal injections of the polysaccharide at an interval of five hours resulted in the arrest of tumor-cell division at metaphase.[1] From the results of our present investigation, it is obvious that repeated administrations of MP5LL4 polysaccharide have no effect on the growth of Ehrlich ascites carcinoma in male Strong A mice. As with the observations of Klein,[24] our data do not bear out the findings of Sugiura,[25] who reported inhibition of the ascitic form of Ehrlich carcinoma by daily intraperitoneal administrations of an older preparation of Serratia marcescens polysaccharide (P-35), although he did note that the substance had no effect on the solid form of this tumor.

Our experimental results suggest that the hosts respond to the administration of MP5LL4 polysaccharide by a production of antibody. This antibody-like substance can be detected in the ascitic fluid, where the neutralization of the viscosity-reducing action of the polysaccharide is readily demonstrable. It is apparent that repeated injections of MP5LL4 polysaccharide, which are theoretically required for prolonging the state of reduced viscosity in tumor cells, result in prompt formation of antibody-like substance by the host. This agent then neutralizes the polysaccharide, and the mitosis-inhibiting activity is therefore nullified.

The inhibition of mitosis by colchicine has been attributed also to the viscosity-reducing action of the cell protoplasm by this alkaloid.[26-28] Colchicine and its derivatives have been reported as prolonging the survival time of mice with Ehrlich ascites carcinoma.[17,29] The effect of repeated doses of colchicine on the growth of Ehrlich ascites carcinoma, with the injections of the drug begun soon after the inoculation of the tumor, can readily be ascertained during the cube-root growth phase of the tumor (Figure). Our data show that multiple injections of colchicine, initially, result in a carcinostatic effect, since at no time is complete absence of tumor cells from the ascitic fluid demonstrated. Furthermore, it is shown that the carcinostatic effect can be overcome eventually if the injections are continued for several days without changing the dosage. This view is in accord with that of Klein et al.[18] The loss of effectiveness of colchicine on repeated administrations in mice with Ehrlich ascites tumor has been observed by others. Lettré and Kramer[30] considered this phenomenon to be the result of drug resistance and have actually developed a strain of N-methyl-colchicamide-resistant Ehrlich tumor cells after 24 transplant generations. Klein, Klein, and Klein,[18] using daily administrations of colchicine for 12 successive transplant generations, were not able to detect evidence of decreased sensitivity of the tumor to colchicine. Without using the prolongation of survival time as a basis for the determination of colchicine resistance, our data show that repeated injections of colchicine result in increased resistance or tolerance by the tumor cells to the drug action as measured by the effect on the protoplasmic viscosity and changes in the mitotic index. When the viscosity values and the mitotic index are used as criteria for comparison, colchicine is shown to have little or no effect on the tumor cells after five to six injections of the alkaloid. If the treated (resistant) tumor cells are transferred intraperitoneally into normal mice and no further colchicine treatment is given during the development of the succeeding generation of tumor cells, then the drug is again found to be effective in reducing the protoplasmic viscosity of the tumor

cells in this generation.[31] The effects of increasing the dosage of colchicine on drug-resistant tumor cells are not known. From these observations and from the effect on the growth curve, it is apparent that tumor cells can overcome the carcinostatic action of colchicine by developing resistance to the drug.

Although MP5LL4 polysaccharide and colchicine were shown to inhibit mitotic division in our previous study, their influence on the growth of Ehrlich ascites carcinoma depends on at least two unrelated phenomena. In the case of the polysaccharide the important factor is the host's production of antibody against it, whereas with colchicine the development of increased drug resistance by the tumor cells appears to be the major reason. No data were collected to determine whether drug resistance was a factor in MP5LL4 polysaccharide treatment or whether antibody response to colchicine ever developed in the hosts.

Summary

Inhibition of cell division may depend on the prevention of mitotic gelation brought about by substances which lower the viscosity of the cell protoplasm. The effect of diminished protoplasmic viscosity of tumor cells on the growth rate of Ehrlich ascites tumor was investigated, using Serratia marcescens polysaccharide and colchicine as viscosity-reducing agents. Repeated intraperitoneal injections of the polysaccharide administered three to five times daily into mice initially inoculated with 2,000,000 tumor cells were shown to have no effect on the rate of tumor growth. On the other hand, one injection of colchicine daily into mice similarly inoculated with the tumor resulted in a pronounced carcinostatic effect, which lasted for 216 hours. After this period, the growth of the tumor was not inhibited effectively by prolonging the administration of the drug.

The evidence presented here indicates that early elaboration of antibody-like substance by the host neutralizes the viscosity-reducing action of Serratia marcescens polysaccharide. In the case of colchicine, the development of drug resistance in tumor cells, after repeated doses, results in a loss of response by the cells to the viscosity-reducing effect of this alkaloid. We therefore believe that complete inhibition of tumor growth cannot be attained with these substances under the conditions in which our experiments were conducted.

Dr. William B. Wartman gave encouragement and suggestions in this investigation.

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Obituaries

CARL VERNON WELLER, M.D.

1887-1956

Carl Vernon Weller, Professor and former Chairman of the Department of Pathology at the University of Michigan Medical School, died suddenly in Ann Arbor on Dec. 10, 1956, of coronary arterial thrombosis, at the age of 69.

He was born in St. Johns, Mich. on Feb. 17, 1887. He received an A.B. degree from Albion College in 1908 and was awarded an honorary Sc.D. degree in June, 1956, by his alma mater. He received an M.D. degree in 1913 and an M.S. degree in 1916, both from the University of Michigan. He was made an instructor in pathology in 1911 and became Chairman of the Department of Pathology in 1931, upon the death of Dr. Aldred S. Warthin. He began his retirement furlough on July 1, 1956.

Dr. Weller married Elsie Huckle in 1913, and they have two sons. Dr. Thomas H. Weller is Strong Professor and Chairman of the Department of Tropical Public Health at Harvard University School of Public Health. Dr. John M. Weller is Assistant Professor of Internal Medicine at the University of Michigan Medical School.

The editorship of *The American Journal of Pathology*, which Dr. Weller had held since 1941, was a continuing project for him at the time of his unexpected death. He was also Chairman of the Scientific Advisory Board of the Armed Forces Institute of Pathology. He had been active in many national medical societies and served as president of the Michigan Pathological Society in 1931,

CARL VERNON WELLER
1887-1956

the American Society for Experimental Pathology in 1933, the International Association of Medical Museums in 1938, and the American Association of Pathologists and Bacteriologists in 1938 and 1939.

He was honored by being invited to deliver the Mellon Lecture in 1941, the Macgregor Memorial Lecture in 1951, and the Beaumont Lecture in 1955. An additional recent honor was the establishment of the Carl V. Weller Lecture by the Michigan Pathological Society. Dr. Howard T. Karsner delivered the first Carl V. Weller Lecture on the subject "The Place of Pathology in Biomedical Research" on Dec. 8, 1956.

Dr. Weller was a great teacher, a tissue diagnostician of wide experience, and a meticulous medical writer and editor. He was respected by students, staff, and colleagues in the Medical School and University as a leader who was devoted to medicine and to the finer aspects of life. His death closed the career of one of the great pathologists of this century.

News and Comment

ANNOUNCEMENTS

Fellowship in Forensic Medicine: Western Reserve University School of Medicine.—Training in forensic medicine is offered by the Department of Pathology of Western Reserve University to qualified persons with a minimum of three years' experience in general pathology. The appointment is for one year, and the period of training is accredited by the American Board of Pathology. The annual stipend is $5000.

The practical work is carried on in the new Cuyahoga County Coroner's laboratories, located on the campus of Western Reserve University, and includes approximately 1200 medicolegal autopsies per year.

Personnel and facilities for training in gross and microscopic pathology, forensic chemistry (toxicology), gross and photomicrography, trace evidence, and forensic immunology are excellent. Fellows will have an opportunity to participate in "on-the-scene" investigation of violent, suspicious, and unexplained deaths. Instruction is provided in the administrative aspects of the modern medicolegal office and legal procedures affecting forensic medicine. An active research program is in progress in several phases of forensic medicine.

Experience in courtroom work is an essential part of the program, and special attention will be devoted to instruction in the presentation of medical evidence in civil and criminal proceedings, both in trial courts and before the Grand Jury and Industrial Commission.

The Cuyahoga County Coroner's Office is an integral part of the Law-Medicine Center of Western Reserve University, where courses are offered throughout the year to attorneys, law students, physicians, medical students, law enforcement officials, etc. Fellows will be encouraged to participate actively in the teaching program.

Course in Ultramicrochemical Methods, Harper Hospital.—The Department of Pathology of Harper Hospital, Detroit, will offer a five-day course in ultramicrochemical methods adapted to hospital laboratory use on April 22 to 26, 1957. This course will be open to a limited number of pathologists, biochemists, residents in pathology, and technologists sponsored by pathologists. Further information may be obtained from Edwin M. Knights Jr., M.D., Department of Pathology, Harper Hospital, Detroit 1.

Oak Ridge Institute of Nuclear Studies: Short-Term Residencies for Physicians.—The Medical Division of the Oak Ridge Institute of Nuclear Studies, in collaboration with hospitals and other medical institutions, provides a three-month residency in Oak Ridge for resident physicians in such fields as medicine, surgery, pathology, and radiology. To provide the best training experience, the ORINS residency should be integrated with the resident's training program and should be taken in the latter part of the residency. Board approval is granted through the parent institution.

The training program for the ORINS residency is flexible and varies with the specialization and interests of the resident. These are examples of the types of programs which have been arranged for residents:

Medicine and Surgery: Participation in care and treatment of research patients; training in use of familiar types of isotope therapy, and new and experimental isotope studies and use of isotopes in diagnostic and tracer studies; may also include laboratory studies and experimental animal research.

Pathology: Assistance in autopsy studies on patients treated with isotopes; training in techniques of handling tissues containing radioactive elements; preparation of gross and microscopic autoradiograms on surgical specimens, autopsy tissues, and animal materials; may also include participation in patient care and treatment.

Radiology: Participation in the clinical radioisotope program and an active teletherapy program using radioisotopes; includes detailed diagnostic x-ray studies with close clinical correlation.

Residents may participate in the four-week basic course in radioisotope techniques offered by the Special Training Division. The usual fee for the course is waived and residents

receive their stipends during the course. Application for the course must be made at least three months in advance on forms supplied by the Special Training Division of the Institute.

Residents are paid an amount equal to the stipend at the time of appointment plus $4.00 per day in lieu of maintenance. The total payment will be at least $180 per month and not more than $350 per month.

The Oak Ridge Institute of Nuclear Studies is a private, nonprofit corporation operating under a contract with the Atomic Energy Commission. The Medical Division operates a medical research program, including basic studies of radioisotopes and therapeutic uses in treatment of cancer and allied diseases; it includes a 30-bed research hospital and well-equipped laboratories for animal studies and related fundamental research.

Residents are accepted upon the recommendation of the chairman of the department or a similar responsible official of the parent institution. Forms for recommending residents are available from The Medical Division, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tenn.

PERSONAL NEWS

Dr. Herwig Hamperl Visiting Professor to University of Wisconsin.—Dr. Herwig Hamperl, director of the Institute of Pathology, University of Bonn, Germany, has been appointed visiting Carl Schurz professor in the University of Wisconsin Medical School, Madison, for the spring semester. Dr. Hamperl will present lectures at the University Medical School and at the University of Wisconsin, Milwaukee.

SOCIETY NEWS

The American Society of Medical Technologists.—The American Society of Medical Technologists will hold its silver anniversary convention at the Palmer House, Chicago, from June 23 to 29, 1957. The program will center around a series of study groups and workshops representative of 25 years of progress. For further details address the Executive Office, #25 Hermann Professional Building, Houston 25, Texas.

Books

BOOK REVIEWS

Clinical Laboratory Diagnosis. By Samuel A. Levinson, M.S., M.D., Ph.D., and Robert P. MacFate, Ch.E., M.S., Ph.D. Price, $12.50. Pp. 1246, with 244 illustrations and 13 plates. Lea & Febiger, 600 S. Washington Sq., Philadelphia, 1956.

This is the fifth edition of a widely used work on clinical laboratory diagnosis. Its objective is to present a suitable review of clinical laboratory diagnosis sufficient to meet the general needs of the medical student, intern, resident, and practicing physician, as well as the medical technologist.

The material is divided according to systems, with a chapter on each part of the alimentary tract, the kidneys, the skin, and many of the body fluids. The consideration of the blood includes chemical procedures, immunology, serology, and hematology. Chapters are included on pediatric procedures, tropical diseases, milk and water analysis, histologic technique, legal medicine, and toxicology.

In general the format remains the same, although the book is enlarged and brought up to date. The entire chapter on hematology is revised. An occasional table has escaped revision, however, such as the one which states that the disturbance in hemophilia is an abnormal thrombocyte disintegration. The chapter on bacteriology has been rewritten from a more practical standpoint. New material has been added in the section on blood chemistry, serology, tropical medicine, and toxicology. Space does not permit listing all of the newer methods included in this edition, but they include many of the procedures in daily use in the institutions with which the authors are associated.

B. H. Spargo, M.D.

Der primäre Leberkrebs. By Kurt Köhn. Price, 15.60 marks. Pp. 85, with 20 figures and 15 tables. Springer-Verlag, Reichpietschufer 20 (1) Berlin W. 35 (West-Berlin); Neuenheimer Landstrasse 24, Heidelberg; Göttingen, 1955.

This little monograph gives an excellent brief review of the subject and presents 85 new cases. Many data from the literature are compiled in tables, and photomicrographs illustrate the main types.

Paul E. Steiner.

Counseling in Medical Genetics. By Sheldon C. Reed. Price, $4.00. Pp. 268. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This book is written to help physicians answer patients' questions concerning the heredity of diseases. About twenty-five chapters are devoted to the diseases and traits that appear with a frequency of more than one per thousand births. In an appendix, information is given on the heredity of many more traits, which are rare. This little book is highly readable and packed with interesting information.

Paul E. Steiner.

PATHOLOGIST

To serve in the newly reorganized modern laboratories of the Medical Examiner's Office of the City of Philadelphia. Duties include supervision of work performed by subordinate pathologists. Prior medico-legal experience not required. Starting salary $13,142 per year. 37½ hour work-week. University or hospital affiliations are encouraged. Qualifications for appointment are eligibility for certification in pathologic anatomy, clinical pathology or both, or recognized sub-specialty, issued by the American Board of Pathology.

Additional information and an application form may be obtained by communicating with Joseph W. Spelman, M.D., Medical Examiner, 13th and Wood Streets, Philadelphia 7, Pennsylvania.

Here are several booklets on mental hygiene that may prove of interest and use to you and your patients.

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James A. Brussel, 6 pages, 10 cents

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George S. Stevenson, 4 pages, 10 cents

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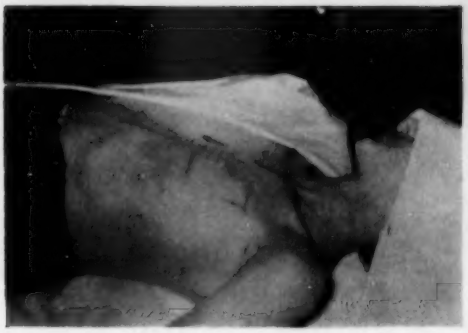

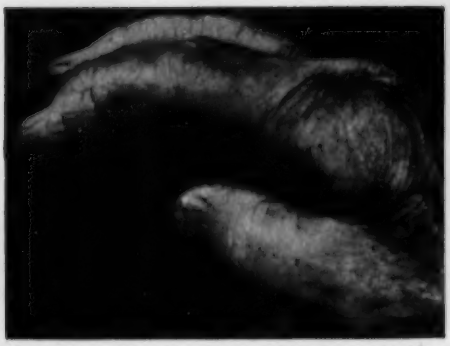

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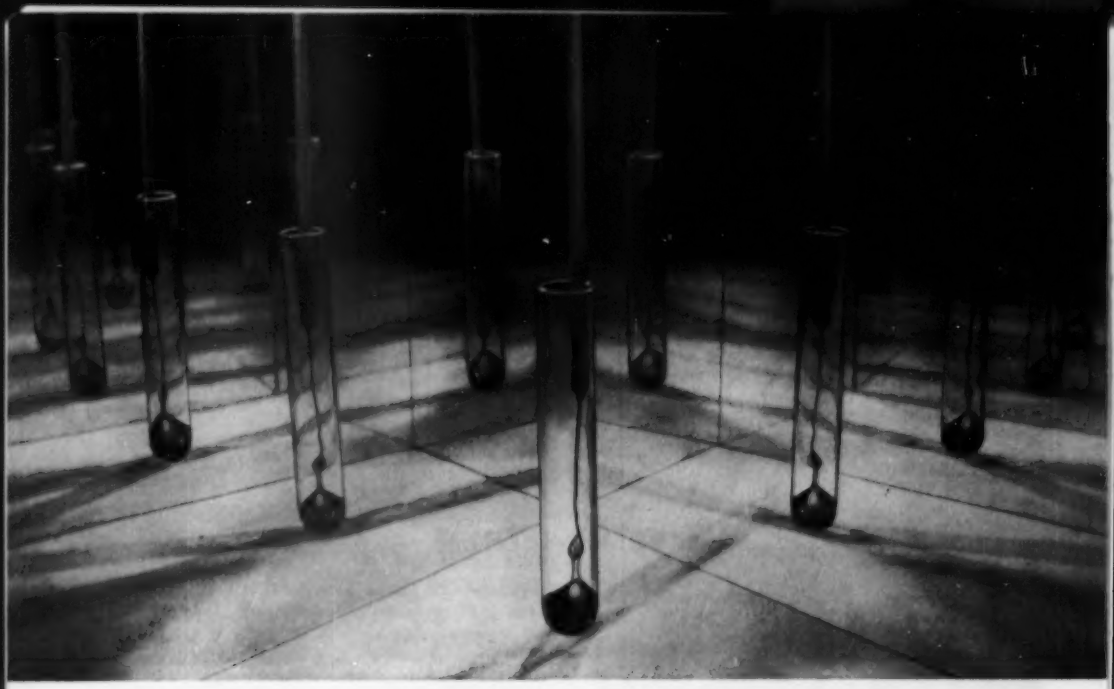

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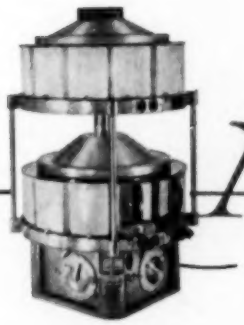

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